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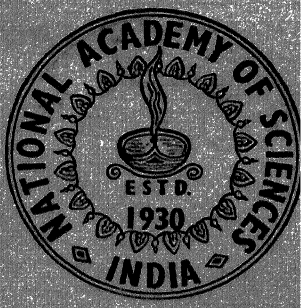

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NITROGEN REQUIREMENTS OF *Fusarium Coeruleum* (LIB.) SACC.

By

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(Received on 11th September 1956)

As nitrogen occupies an important place in fungal nutrition, study of the nitrogen requirements of fungi has attracted various workers. A large amount of literature has accumulated on the subject and it is evident from its perusal that all nitrogen sources are not equally suitable for different fungi. The fungi have definite specificity for the nitrogen compounds they can utilize. Robbins (1937) and Steinberg (1950) have classified the fungi according to their ability to utilize different sources of nitrogen. The four main groups suggested by Robbins are as follows :—

1. Fungi utilizing nitrate, ammonium and organic nitrogen and are capable of fixing elemental nitrogen also.
2. Fungi utilizing organic nitrogen, ammonia and nitrate nitrogen but are unable to fix elemental nitrogen.
3. Fungi utilizing organic nitrogen and ammonia.
4. Fungi utilizing organic nitrogen alone.

Mix (1953) had reported that all species of *Taphrina* showed a characteristic pattern of nitrogen utilization. Some host forms within the species agreed closely in behaviour while others showed various degrees of difference. The present investigation deals with the nitrogen requirements of the two strains of *Fusarium coeruleum* isolated from potato and *Colocasia antiquorum*.

MATERIAL AND METHODS

Fusarium coeruleum was isolated from diseased potato and *Colocasia antiquorum*. Single spore cultures were prepared by the usual method. Asthana and Hawker's medium A containing 5 gm glucose, 1·75 gm., KH_2PO_4, 0·75 gm., $MgSO_4.7H_2O$, 3·5 gm., KNO_3 and 1 litre water was used as the basal medium. In order to study the effect of various nitrogen compounds they were singly substituted for KNO_3 of the basal medium. The quantity of different compounds was so adjusted as to contain an amount of nitrogen equivalent to that present in 3·5 gm. of KNO_3.

Throughout the experiment only guaranteed reagents, Pyrex glasswares and doublly distilled water, were used. Liquid cultures containing 50 c.c. of the medium were taken in 150 c.c. conical flasks. The pH of all the media, after adding different nitrogen compounds, was adjusted to 6·4, which was found to be most suitable on the basis of previous investigations. Four replicates were used for each treatment.

The solutions were autoclaved at 15 lbs. pressure for 15 minutes and after inoculation they were incubated at room temperature for 15 days.

Following nitrogen compounds were tried :

I. Inorganic compounds

Potassium nitrate, sodium nitrate, ammonium nitrate, ammonium sulphate, ammonium chloride, sodium nitrite, hydrazine hydrochloride.

I. Organic compounds

(*a*) Monoamino monocarboxylic acids
1-phenyl-alanine, glycine, d-alanine

(*b*) Monoamino dicarboxylic acids
1-aspartic acid

(*c*) Amides of monoamino dicarboxylic acid
1-asparagine

(*d*) Basic aminoacids
Histidine

(*e*) Acid amides
acetamide

(*f*) Amines
urea

(*g*) Proteins
peptone

(*h*) Sulphur containing amino acid
cystein

OBSERVATIONS

The dry weights of the two strains after the growth on various media are recorded in Table I.

TABLE I

Showing the dry weight of the two strains of *Fusarium coeruleum* on media containing equivalent quantities of different nitrogen compounds.

No.	Nitrogen compounds	Dry wt. in mgms.	
		F. coeruleum from potato	*F. cocruleum from* C. *antilquorum.*
1.	Acetamide	94·4	113·4
2.	Potassium nitrate	90·2	146·0
3.	Asparagine	87·6	96·2
4.	Aspartic acid	82·0	116·4
5.	Glycine	81·4	110·0
6.	l-phenyl-alanine	77·4	112·2
7.	d-alanine	70·0	106·0
8.	Peptone	66·2	92·2
9.	Sodium nitrate	64·8	100·0
10.	Ammonium nitrate	63·4	82·4
11.	Histidine	62·2	82·0
12.	Cystein	58·0	76·0
13.	Ammonium sulphate	49·6	65·2
14.	Ammonium chloride	46·2	53·0
15.	Sodium nitrite	41·4	112·6
16.	Urea	40·0	77·6
17.	Hydrazine hydrochloride	31·0	39·6
18.	No nitrogen	0·0	0·0

Summary of dry weight results and conclusions at 5% level of P.

S.E.	C.D. at 5%
3·4	9·5

Dry weight results of the potato strain

Nos. 1 2 3 4 5 6 7 8 9 10 11 12 13 14

15 16 17

Dry weight results of Colocasia strain

Nos. 2 > 4 1 15 6 5 7 9 3 8 > 10 11 16 12 > 13 > 14 > 17

It is clear from the above table that the growth of the *Colocasia* strain on various nitrogen compounds used in the present investigation is significantly better than that of the potato strain. The dry weights recorded in the above table show that significantly good growth of the potato strain was observed on acetamide, potassium nitrate, asparagine, aspartic acid, glycine and l-phenyl alanine. The differences in growth between acetamide, potassium nitrate and asparagine were statistically insignificant. Similarly there was no statistical difference between potassium nitrate, asparagine, aspartic acid and glycine. The growth on aspartic acid, glycine and l-phenyl-alanine was statistically similar and was less than that on acetamide.

Moderate growth of the potato strain was recorded on d-alanine, peptone, sodium nitrate, ammonium nitrate, histidine and cystein. Statistically there was no difference in the growth on the above compounds except on d-alanine on which the growth was more than that on cystein.

Significantly poor growth of the above strain was recorded on ammonium sulphate, ammonium chloride, sodium nitrite, urea and hydrazine hydrochloride. The growth on ammonium sulphate and ammonium chloride was similar. Statistically there was no difference in the dry weights of ammonium chloride, sodium nitrite and urea.

Good growth of the Colocasia strain was obtained on potassium nitrate, aspartic acid, acetamide, sodium nitrite, l-phenyl-alanine, glycine, d-alanine and sodium nitrate. There was no significant difference in the growth between aspartic acid, acetamide and sodium nitrite but it was less than that on potassium nitrate. The differences in growth between acetamide, sodium nitrite, l-phenyl-alanine, glycine, and d-alanine were also statistically insignificant. The growth on sodium nitrate was similar to that of d-alanine but it was less than that on any other compound mentioned above.

The Colocasia strain supported moderate growth on asparagine, peptone, ammonium nitrate and histidine. The growth on asparagine and peptone was statistically similar and was more than that recorded on ammonium nitrate and histidine.

The above fungus supported poor growth on urea, cystein, ammonium sulphate, ammonium chloride, and hydrazine hydrochloride. There was no difference in growth between urea and cystein. Growth on cystein was more than on ammonium sulphate which was better than ammonium chloride. Hydrazine hydrochloride gave least growth amongst the compounds which supported poor growth. The two strains could not grow in the complete absence of nitrogen.

It is clear that the two strains differed in their likings for the different sources of nitrogen. Potassium nitrate supported the best growth of the Colocasia strain while acetamide was most suitable for the potato strain where potassium nitrate and asparagine were statistically similar. Asparagine supported good growth of *F. coeruleum* from potato though it was only a moderate source for Colocasia strain. d-alanine, sodium nitrate and sodium nitrite supported good growth of *F. coeruleum* from Colocasia while the potato strain showed moderate growth on d-alanine and sodium nitrate and poor on sodium nitrite. Significantly good growth of the Colo-

casia strain on sodium nitrite is interesting as many fungi do not grow on it. Cystein supported moderate growth of potato strain and poor of the other strain.

DISCUSSION

In the present investigation good growth of the two fungi was recorded on potassium nitrate. Similar results were obtained by Tandon and Grewal (1956). Subramanian and Srinivasa Pai (1953), Matsumato (1921), Neal et al (1933), Uppal etal (1938), Leben and Keitt (1948) and Srivastava (1951) for *Gloeosporium* sp. and *Colletotrichum papayae*, *Fusarium vasinfectum*, *Rhizoctonia*, *Phymatotrichum omnivorum*, *Alternaria burnsii*, *Venturia inaequalis* and *Alternaria tenius* respectively. Fergus (1952) working on *Penicillium digitatum* found that nitrates (with the exception of (NH_4NO_3) were either not at all utilized or were only very poorly utilized. Wooster and-Cheldelin (1945), however, found that potassium nitrate was a poor source for the growth of *Penicillium digitatum*. Sodium nitrate supported good growth of *F. coeruleum* isolated from Colocasia though it was only a moderate source for the potato strain. Neal et al (1933) and Grewal (1954) obtained good growth of *Phymatotrichum omnivorum*, and *Gloeosporium musarum*, *G. papayae*, and *Colletotrichum papayae* on sodium nitrate but according to Tochinai (1926) it supported poor growth of *Fusarium lini* while *Rhizopus oryzae* investigated by *Lockwood* et al (1936) was unable to use it. Ammonium nitrate supported moderate growth of the two strains of *F. coeruleum*. The result of Grewal (1954) for *Alternaria tenuis*, *Gloeosporium musarum* and *G. papaye* were similar, but Tochinai (1926), Neal et al (1933) and Wooster and Cheldelin (1945) found it to be a poor source of nitrogen for the fungi investigated by them. The growth of both the organisms was poor on ammonium sulphate and ammonium chloride. Similar results with ammonium sulphate were obtained by Patel et al (1950) and Neal et al (1933) for *Pestalotia psidii* and *Phymatotrichum omnivorum* respectively. Subramanian and Srinivasa Pai (1953) reported that ammonium sulphate was a poor source of nitrogen for vegetative growth of *Fusarium vasinfectum*. Haeskaylo, Lilly and Barnett (1954) found that ammonium sulphate restricted the growth of most species studied by them but Wolf (1953) observed that it supported good growth of *Ustilago zeae*. Tochinai (1926), Srivastava (1951) and Tandon and Grewal (1956) also obtained poor growth of their fungi on ammonium chloride. The results obtained by Mix (1952) established that *Taphrina americana* and *T. caerulscens* were unable to use nitrogen from ammonium chloride.

Hydrazine hydrochloride supported only poor growth of the two strains of *F. coeruleum*. Tandon (1950) working with *Pestalotia malorum* and *P. psidii* as well as Brock (1951) with *Morchella esculenta* obtained no growth on hydrazine hydrochloride.

Though generally nitrite nitrogen is considered toxic to fungi but Lockwood et al (1936), Leonian and Lilly (1938), Talley and Blank (1941), Ramakrishnan (1948), Brock (1951), and Marsden (1954) had reported that *Rhizopus oryzae*, *Blakeslea trispora*, *Phymatotrichum omnivorum*, *Piricularia* sp., *Morchella esculenta* and *Hormodendrum vesinae* could grow on nitrites. The present results confirm their observations and add the two strains of *F. coeruleum* to the list of fungi which grew on nitrites. In fact the Colocasia strain had fairly good growth on sodium nitrite. Brock (1951) has suggested that the toxicity of nitrites may be associated with pH range specially because most of the fungi are cultured in the acid range where nitrites are reported to be toxic by Cochrane (1950) and Cochrane and Conn (1950). He has further poirted out that *Morchella esculenta* could develop well on nitrites because it grows best when the reaction of the medium is near about the neutral point. In the present investigation, however, it was found that *F. coeruleum* could grow well even when the

pH of the medium was 6·4 (i. e. even when the medium was acidic). A more detailed investigation of the role of pH in the utilization of sodium nitrite by *F. coeruleum* was undertaken by Tandon and Agarwal (1953) . They showed that the two strains of *F. coeruleum* could grow in fairly acidic media (pH4 for Colocasia strain and 4·4 for the potato strain) but whenever growth took place in an acidic medium the final reaction became markedly alkaline. Cochrane (1950) and Cochrane and Conn (1950) have reported that nitrites were toxic in the acid range but Tandon and Agarwal (1953) have suggested that the marked growth of the two strains of *F. coeruleum* under those conditions may be due to the activity of these organisms which changed the medium from acidic to alkaline range. They found that when the initial pH was only 4 the potato strain modified the final pH to 5·6 only, and no marked growth of that strain was noticed though the other strain modified the pH to 8.8 and it showed a good growth. They concluded that in some cases it may be possible to observe good growth on fairly acidic media but it seems that the good growth appears only after the medium becomes alkaline. They also suggested that the nitrites do not kill the organism at the acid range but merely inhibit the growth which becomes possible after the medium becomes alkaline.

Glycine supported good growth of the two strains of *F. coeruleum*. Similar results were obtained by Steinberg (1942), Gottlieb (1946), Wolf et al (1950) and Converse (1953) for *Aspergillus niger*, *Fusarium oxysporum*, *Monosporium apiospermum* and *Helminthosporium gramineum* respectively but Wooster and Cheldelin (1945), Wolf (1945), and Brock (1951) obtained poor growth of the fungi studied by them. Wolf and Shoup (1943), however , observed that all species of Allomyces tried by them were unable to assimilate glycine.

Good growth of the Colocasia strain was obtained on d-alanine but the growth of the potato strain was found to be moderate. Steinberg (1942), Srivastava (1951) and Wolf (1953) have reported that d-alanine supported good growth of *Aspergillus niger*, *Alternaria tenuis*, and *Ustilago zeae* but Lockwood et al (1936), Wolf (1949), and Wolf et al (1950) obtained only moderate growth of their fungi. l-Phenyl alanine supported good growth of the two strains of *F. coeruleum*. Similar results were obtained by Gottlieb (1946), Wolf (1949), Srivastava (1951) and Grewal (1954) for the fungi investigated by them. Mosher et al (1936), Steinberg (1942), and Wolf et al (1950), however obtained only poor growth of their fungi on l-phenyl alanine. The results obtained with aspartic acid were similar to those of Mosher et al (1936), Steinberg (1942), Brock (1951) and Srivastava (1951), but they differed from those of Tandon and Grewal (l.c.) who obtained poor growth of *Gloeosporium musarum*, *G. papaye*, and *Colletotrichum papayae*.

Spermophthora gossypii, *Nematospora coryli* and *N. gossypii* investigated by Farries and Bell (1930) could not grow on asparagine but it was found to support good growth of the potato strain of *F. coeruleum*. Similar results were obtained by Tochinai (1926), Mosher et al (1936), Wooster and Cheldelin (1945), Gordon (1950), Patel et al (1950), Brock (1951), Srivastava (1951) and Wolf (1953) etc., for the fungi investigated by them. *F. coeruleum* from Colocasia developed only moderate growth on asparagine. The growth of both the strains was moderate on histidine which supported good growth of *Ustilago zeae* Wolf (1953) and *Gloeosporium* sp. (Grewal 1954) while Steinberg (1942), and Brock (1951) obtained only poor growth of *Aspergillus* niger and *Morchella esculenta*.

The two fungi used in the present investigation supported good growth on acetamide and in this aspect they were similar to the fungi investigated by Srivastava

(1951) as well as Tandon and Grewal (1956) but Brock (1951) obtained only poor growth of *Morchella esculenta*. Ajello (1948) reported that *Polychytrium aggregatum* was unable to use urea which supported poor growth of the two strains of *F. coeruleum*. The present results were similar to those of La Fuge (1937), Uppal et al (1938), Wooster and Cheldelin (1945) and Srivastava (1951). The growth on peptone was moderate even though it supported poor growth of *Alternaria tenuis* investigated by Srivastava (1951).

It is obvious from the present investigation that the two strains of *F. coeruleum* show differences between themselves but they can utilize nitrates, ammonium salts and organic sources of nitrogen. They are unable to fix the atmospheric nitrogen. They should, therefore, be placed in Group 2 of the classification proposed by Robbins (1937).

SUMMARY

The growth of the two strains of *F. coeruleum* isolated from potato and *Colocasia antiquorum* on different nitrogen compounds has been studied. The two fungi could not grow in the complete absence of nitrogen from the medium. Potassium nitrate supported the best growth of the Colocasia strain while acetamide was most suitable for the potato strain. The two fungi could grow on sodium nitrite which supported good growth of the Colocasia strain but poor of the potato strain. The poorest growth of the two organisms was on hydrazine hydrochloride. Both the strains of *F. coeruleum* have been placed in Group 2 of the classification proposed by Robbins (1937).

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OBSERVATIONS ON THE FEEDING HABITS AND DIGESTION IN THE LARVA OF *Dacus cucurbitae* COQUILLETT (DIPTERA : TRYPETIDAE)

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(Received on 13th October, 1956)

Dacus cucurbitae is a serious pest of cucurbitaceous fruits, although it has been reported occasionally from fruits of other families as well.

The adult fruit fly does not cause any damage to the crop by feeding, since it only rasps nectar from the flowers. But its female ruptures the epicarp of fruits with its ovipositor and deposits the eggs in the mesocarp with the micropyle pointing towards the endocarp. Young, soft and juicy fruits are preferred for this purpose to older fruits with hard epicarp. On hatching, the larvae feed on the pulp and gradually move inwards in the fruit, all the time passing their excreta inside the fruit. When most of the pulp has thus been eaten away, the larvae again move towards the surface and finally migrate to other fruits in search of more food or into the soil for pupation.

Normally, eggs are not laid in older fruits ; but in the bitter gourd, luffah and snake cucumber, in which the epicarp does not become particularly hard even in advanced fruits, eggs may be laid, in all except in the very old stages. In the absence of suitable cucurbitaceous fruits, eggs may be laid in ripe tomatoes as well.

The fact that the pest is attracted specially to cucurbits, and its preference for *Momordica charantia* and *Cucumis melo* var. *utilissimus* in May and June, when several cucurbits are grown side by side, shows the existence of well developed senses of smell and taste in the pest.

DETERMINATION OF THE HYDROGEN-ION CONCENTRATION

In view of the very small quantity of secretion available in the different parts of the gut, and failure to feed the larvae on indicators, pH comparator and pH metre methods were abandoned in favour of indicator paper method for determination of pH.

The larvae were starved for four to six hours to clear their guts of the food contents to a considerable extent, and then dissected in their own haemolymph. The salivary glands and the different parts of the alimentary canal were separated, gut content, if any, removed with a micropipette, and the inner wall of the gut teased gently and brought in contact with the indicator papers (Merck). The pH of the peripheral and central regions of important host fruits (from portions in which egg laying and feeding had not taken place) was also similarly determined. The results obtained are given in Tables 1 and 2.

TABLE 1

pH of different parts of the digestive system.

Region	pH
Haemolymph	6·8
Salivary gland	6·2
Foregut	6·4
Midgut	6·6
Hindgut	6·4
Excreta	5·4—5·7

TABLE 2

pH of the peripheral and central regions of important host fruits.

Name of fruit	Region of fruit	
	peripheral (egg laying) region	central (feeding) region
Momordica charantia (bitter gourd)	6·4	6·2
Lagenaria vulgaris (bottle gourd)	6·8	6·0—6·2
Trichosanthes anguina (snake gourd)	6·6	6·2
Cucumis melo var. *utilissimus* (snake cucumber)	6·4	6·0
C. melo (musk melon)	6·4	6·0—6·2
C. sativus (cucumber)	6·6	6·2
Citrullus vulgaris (water melon)	6·4	6·2
C. vulgaris var. *fistulosus* (stocks)	6·6	6·0
Cucurbita pepo (pumpkin)	6·4—6·6	6·0
Luffa acentagula (ribbed gourd)	6·4—6·6	6·0
L. aegyptica (luffah)	6·6—6·8	6·2

The data in Table 1 show a clear rise of pH from the foregut to the midgut and then a fall again in the hindgut. Table 2 indicates that a small but distinct difference exists in the pH of the egg laying and feeding regions of the host fruits, the pH of the latter invariably being lower than that of the former. It appears relevant that the pH of the feeding region in the different host fruits examined is fairly similar, varying between the narrow limits of 6·0 and 6·2. The two regions also differ in their moisture content, the outer region having less water. But the percentage of water in different cucurbitaceous fruits appears to differ.

QUALITATIVE DETERMINATION OF ENZYMES

The larvae were chloroformed and dissected immediately on a depression slide

in a few drops of distilled water. The salivary glands and the different parts of the alimentary canal were removed from other tissues, separated and washed in cold distilled water. Each part was then ground separately with glycerine. Any suspended tissue in the extract thus prepared was removed and then it was transferred to a tube. A few drops of toluene were also put in the tube to prevent access of micro-organisms. In view of the small size of the gut, sections of the gut of several insects were usually taken together for preparing the extracts.

Different qualitative tests for the detection of the enzymes amylase, maltase, invertase , lactase, lipase and proteases were performed with each extract at a temperature of 37°-38° C. Each test was accompanied by a control test performed with boiled tissue suspensions of the same extract incubated under the same conditions as the test tissue suspensions. Distilled water used during the entire work was freshly boiled and cooled before use.

The tests performed, together with the results obtained are given in Table 3. Confirmatory tests and modifications, if any, are presented later. Since no enzyme was detected in the foregut and hindgut, tests with their extracts have not been included in the table.

TABLE 3

Qualitative assay of the enzymes in the alimentary canal of the larva of *D. cucurbitae.*

Enzyme tested for	Tests performed	Substrate used.	Reaction with extracts of	
			Salivary gland	Midgut.
1. Amylase	Potassium iodide Iodine test (H. S. Swingle, 1928)	0·5% boiled soluble starch	+	+
2. Maltase	Osazone test (H. S. Swingle, 1928)	15% maltose solution	..	+
3. Invertase	Fluckiger test (H. S. Swingle, 1928)	15% sucrose solution	..	+
4. Lactase	Osazone test (H. S. Swingle, 1928)	15% lactase solution.	..	+
5. Lipase	Brom-thymol test (M. C. Swingle, 1930)	10% condensed milk solution	..	+
6. Protease	Albumen test (Hinmann, 1933)	Coagulated egg albumen	..	+

The presence of amylase was further confirmed by picramic acid test and Fluckiger test with the incubated tissue suspensions already subjected to the Potassium

iodide-Iodine test. The tests gave positive results, showing the presence of reducing sugar, formed obviously by the hydrolysis of starch by amylase.

The presence of maltase was confirmed by Barfoed's test and Fehling's test.

In the test for lipase, instead of a 0·1% solution of brom thymol blue, a 1·0% solution (4-6 drops) was mixed with 25 ml. of 10% condensed milk solution and 1% KOH solution added until the solution turned blue. Equal volumes of this solution and the extract were incubated together for 48 hours.

Tests for proteases were performed at different hydrogen-ion concentrations (6·4 and 8·5-9·0). It was found that the enzymes acted equally well under both conditions.

Discussion

A number of outstanding points emerge from the observations in this insect.

It is well known that most insects, except for certain phytophagous ones, show preference in feeding and Trager (1948) has shown that the choice of food is generally determined by the nature of the food and not directly by the nutritional requirement of the particular insect. The selection of its food by the larva of *D. cucurbitae* seems to be influenced by no less than three factors, viz., the age, odour, and pH value of the fruits. It is noted that the larvae are restricted mainly to fruits of the Cucurbitaceae and show preference to *Momordica charantia* and *Cucumis melo* var. *utilissimus* in May and June when several cucurbits are grown side by side. This proves that the insects, have a well developed sense of smell. Again, younger fruits are preferred to older ones for egg laying purpose, showing the preference of larvae to the former as food, except in bitter gourd, luffah and snake cucumber.

It is observed that the larvae emerging from the eggs laid in the peripheral region of the fruits at once migrate to the more central region. In the light of the observation that the pH in these two regions of cucurbitaceous fruits differs distinctly, we are lead to conclude that the pH of the food may be an important factor determining its choice. The pH in the peripheral region of different cucurbitaceous fruits is generally above 6·2, while in the central region it is 6·0 to 6·2 When the larvae have settled down for some time, eating and passing their excreta in the pulp, the pH may go up to 7·0 or 7·2. In such a situation, the larvae refuse the food available and move deeper in a region where the pH is lower. It is thus clear that the larvae exhibit a discrimination in their food, going in for food within a definite pH range. In other words, we might state that wherever the food substance of an insect shows a gradient in respect to the hydrogen-ion concentration, the insect can find the region of most suitable pH and commences to feed there. There are two other possible reasons for the preference shown by the larvae to the inner region of the fruit: greater moisture content and better food value of the material in the central region. With regard to the first, one may state that the moisture content of different host fruits in the two regions respectively is not uniform and the percentage of water in the pripheral region of one fruit may be the same as in the central region of another. Obviously, therefore, the preference for the central region cannot be attributed to the percentage of water only. Chemical analysis of the substance in the two regions of different cucurbitaceous fruits has not been done but, as stated earlier, nutritional value of the food may hardly be responsible for its preference. Above all, the fact that when the pH of the central re-

gion rises, larvae migrate from there also, proves that the pH is an important factor determining the preference.

As regards the pH in the larval gut, we note a rise and fall in it as we proceed from the foregut to the midgut and then to the hindgut. The pH in the midgut region is fairly constant at 6·6, and, therefore, distinctly higher than that of the food substance. Obviously, the midgut pH does not depend on the pH of the food. This view has been established previously by several workers. Swingle (1931), for example, did not note any change in the pH of the gut of *Popillia japonica* larvae by feeding them on samples of soil of varying pH. Such a constancy of midgut pH and its relative independence of the pH of the food points to the presence of some kind of buffer mechanism in the midgut.

As has been established in other insects, in *Dacus* larvae also enzymes are secreted only by the salivary glands and the midgut. The presence of amylase in the salivary gland and midgut of the larva indicates the ability of hydrolysing and utilising starch. Likewise, the detection of maltase, invertase and lactase demonstrates that the larva can hydrolyse maltose, sucrose and lactose. The presence of lipase and protease proves the capacity of the insect for the digestion of fats and protein. Hering (1926), Hinmann (1933) and Bramstedt (1948) have also concluded that protein is completely utilised along with soluble sugars, and Brown (1937) has demonstrated complete absence of protein from the excreta of *Melanoplus bivittatus*. It is interesting that the digestion of protein in *Dacus* larvae occurs both in acidic, as well as, alkaline media. It is possible that the enzyme responsible is active on both sides of neutrality, as noted by Wigglesworth (1953) in the cockroach and blowfly larva or that there are two distinct enzymes, each acting in a particular region of the midgut. Such regional difference in the midgut pH has also been reported by Wigglesworth (1953) in larvae of *Tenebrio* and larvae and adults of *Lucilia*. Unfortunately, it has not been possible to detect localised differences in the pH with the paper indicator method and, at this stage, we are not in a position to say which of the two possibilities may account for the phenomenon.

Summary

Dacus cucurbitae lays its eggs beneath the epicarp in the peripheral region of the cucurbitaceous fruits and occasionally in tomato.

On hatching, the larvae move to the central region of the fruit. This habit seems to be correlated with the difference in the pH of the different regions of the fruit, the pH of the peripheral region being slightly higher (6·4—6·8) and that of the central region lower and more or less constant (6·0—6·2) in all cucurbitaceous fruits.

The pH of the different regions of the alimentary canal of the larva has been determined. It differs in the different sections of the gut. The midgut pH is not affected by the pH of the food.

The salivary glands secrete amylase and the midgut amylase, maltase, invertase, lactase, lipase and protease.

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ON THE CYTOPLASMIC INCLUSIONS IN THE OOGENESIS OF BIRDS : THE PHENOMENON OF INFILTRATION

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It has long been thought that the epithelium of a growing oocyte, besides affording a means of protection, supplies the egg-cell with nutritive material. It was, however, Bhattacharya who in 1925, for the first time, observed in the egg-cells of tortoises that the cytoplasmic inclusions passed out from the follicular epithelium and added themselves to the bulk of the elements in concentration at the periphery of the oocyte. The idea that some of the immense number of Golgi bodies present in the extreme cortical region of the egg were possibly derived, at least partly, from the follicular epithelium also occurred to Brambell in the same year while he worked on the fowl, *Gallus bankiva*. Since then this phenomenon has been observed by workers in this laboratory and elsewhere in various animals, both vertebrate and invertebrate.

An attempt to study this interesting phenomenon in the sexual cells of some birds was made and the results herein incorporated further confirm the idea that infiltration phenomenon is an actuality. The results obtained here are in line with the findings of Bhattacharya and Brambell.

This work was carried out in the laboratory of the Zoology Department of Allahabad University. The author takes this opportunity to express his gratefulness to late Professor D. R. Bhattacharya whose advice and interest were invaluable.

MATERIAL AND TECHNIQUE

For the purpose of the present study five species of birds, *Accipiter nisus nisus* (Fam. *Falconidae*), *Hoplopterus ventralis* (Fam. *Charadrudae*), *Ardeola grayii* (Fam. *Ardidae*), *Turdoides terricolor terricolor* (Fam. *Turdoideae*) and *Acridotheres tristis tristis* (Fam. *Sturnidae*) were used. All these birds were caught locally at Allahabad, its deep country side and the nighbouring jungles in the district of Mirzapur. Severing off of the neck, to out open the wind-pipe and the main blood vessels of the neck, was the usual method of killing these birds as chloroform and other anaesthetics, for this purpose, were avoided. The ovarian pieces were then transferred to the usual cytological fixatives directly or were first put in Ringer's salt solution and then placed in the fixatives. The whole process, from the killing of the birds to the placing of the ovarian tissue into fixatives, did not take more than just a couple of minutes and thus , all chances of post-mortem change were reduced to the barest minimum. Pinching of the tissue by forceps was also avoided. In every case all precautions, as recommended in Bolles Lee's Vade Mecum, were kept in view.

Cajal, da Fano, Aoyama in silver fixatives, Regaud-tupa, Zenker-Helly in chrome-formol, and chrome-osmium fixatives like Ludford's modification of Mann-Kopsch fixative and F. W. A. gave better results and, therefore, these fixatives were extensively used. In all these fixatives the infiltration phenomenon was well observed.

For furhter demonstration of the phenomenon of Golgi infiltration the following modification of da Fano, as recommended by Das (1930) was usefully and succegsfully employed :

Uranium nitrate 1 gm
Cobalt nitrate 1 gm
Distilled water 100 c.c.
Neutral formol 40 per cent. 5 c.c.

The subsequent procedure remained exactly the same as in other silver fixatives except for the reduction in the original duration of the fixing period which was reduced from twenty-four to eighteen hours.

Again, the following fixative, as modified by the author, was not only successfully used for the study of the Golgi elements but also gave good results towards the study of the phenomenon of infiltration of this element :

Cobalt nitrate ·5 gm
Cadminum chloride ·5 gm
Distilled water 100 c.c.
Neutral formol 40 per cent 5 c.c.

The subsequent procedure continued to be as in any other silver fixative and the fixation period also in this case continued to be the usual twenty-four hours.

In all these cases the quantity of neutral formalin (40%) recommended in Lee's Microtomist's Vade Mecum, proved unsuitable as it resulted in extreme shrinkage in the egg-cells. Consequently , the quantity of formalin had to be reduced for a successful study of the oocyte in birds (Varma, 1942). Sections of the paraffin embedded materials were cut by means of microtome at five microns and sections were mounted finally in neutral canada balsam.

OBSERVATIONS

While studying the animals under examination in detail from the cytological view point it was observed that in both osmic as well as non-osmic preparations the oocytes at a particular stage displayed profusely and most beautifully the phenomenon of infiltration of both Golgi bodies and mitochondria. These inclusions were seen to come into the oocyte proper from the follicle cells. The follicular epithelium layer cells as well as the extreme periphery of the oocyte at this stage in the life of the oocyte are seen studded with both of these inclusions and their passage from the former to the latter can best be traced after the silver methods, specially by Das's method and the new formula mentioned earlier, which is a combination of uranium nitrate and cadmium chloride, and also by osmic preparations specific for the demonstration of Golgi bodies and mitochondria respectively.

In the course of the present study the phenomenon of infiltration, as seen in the silver preparation, were always confirmed by the osmic methods as well.

The occurance of Golgi bodies in the follicular epithelium was extensively seen during the course of the present work. Quite a large number of the Golgi elements are seen to be indiscriminately distributed around the nucleus in the follicular epithelium and extreme cortical region of the oocyte in irregular masses (Fig. 1). This happens when the epithelial layer has attained a two or three-layered condition and there is still no trace of the zona radiata although the limiting membrane is easily discernible. This is the period of utmost activity for the follicular epithelium of the egg. Prior to their exurusions the Golgi elements seem to mass together on the border adjacent to the envesting egg membrane. This stage is preparatory to the next when the Golgi elements are extruded from the follicular epithelium to the egg. At places it can very well be seen that the Golgi granules are lying half-way inside the follicular epithelium and the other half in the periphery of the egg proper; while at other places it may be seen that these granules are coming out in great numbers at of places establishing clearly a connecting link between the epithelial layer and the cortical region of the oocyte. It decreases in intensity as the egg grows and then the process of infiltration is confined to a number of regions or patches. All stages of descent from the epithelium to the egg can easily be made out under an oil immersion lens. Both the cell-membrane of the follicular layer and the demarcating membrane of the oocyte are so thin that they offer no resistance to the infiltrating Golgi bodies. Another noteworthy feature is that the Golgi mass present in extreme periphery of the egg-cell as well as those occuring inside and at the bases of the follicle cells are exactly alike in shape, structure and nature. The actual infiltration of the Golgi elements in the birds under study occurred haphazardly and at random in granular form and not in lumps as observed by Brambell in *Gallus bankiva*. The infiltrating Golgi elements have nothing particular to demarcate them from those that are either in the follicle cells or the oocyte proper. After infiltration they get mixed up with those of their kind in the egg cell itself situated at the extreme cortical region of the egg in the form of a definite and regular band. This peripheral layer, owing to this accumulation of the Golgi granules, becomes dark and of considerable thickness (Fig. 1) against a clear or stained background of the cytoplasm. Consequently it establishes the fact that the formation of the peripheral band of the Golgi elements in cells, partly at least, is due to the infiltration phenomenon. The passage of Golgi bodies in two stages or instalments, as in the fowl (Brambell, 1925) or the pigeon (Das, 1931) or their transference through canalicular channels, as mentioned in tortoises (Bhattacharya, 1925), could not be noticed in any of the five species of the birds examined. Finally, this phenomenon of the infiltration of Golgi bodies, it seemed, ceased in very advanced oocytes.

It was noticed during the course of the present work that the follicular cells, as was the case with the Golgi bodies, also was profusely packed with fine granular form of mitochondria. These numerous and fine granules were seen to be lying on both the sides of the follicle cells both in patches and isolated forms facing, on the one hand the oocyte proper, arranged excentrically in the cell pointing towards the end nearest to the oocyte, and on the other, the thecal layer (Fig. 2). Further, it is seen at places they establish a continuity by means of these fine granules, between those that are in the epithelial layer and those of their kind already deposited at the periphery through the limiting membrane of the oocyte. Some of these granules, like the Golgi ones, were seen to be actually lying half way in the follicular epithelium and the cortex of the oocyte itself. Before the formation of the zona radiata the mitochondrial infiltration is rather profuse although with its appearance there seem

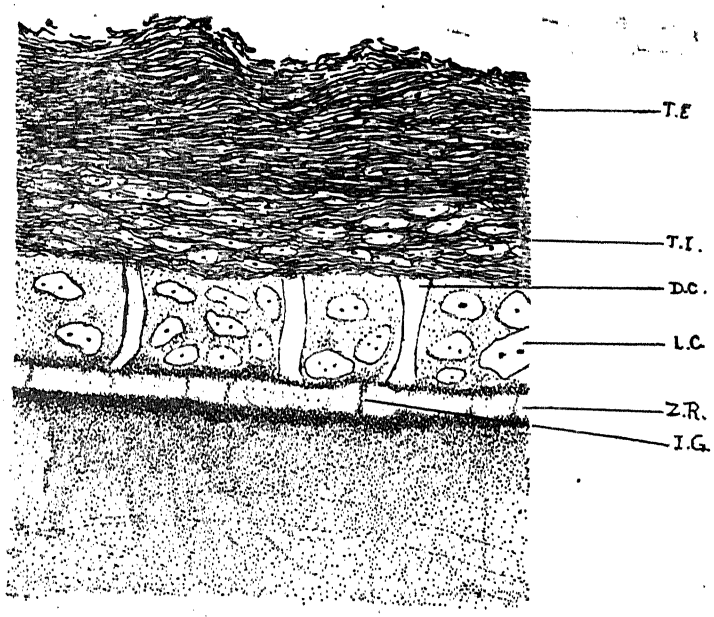

Fig. 1

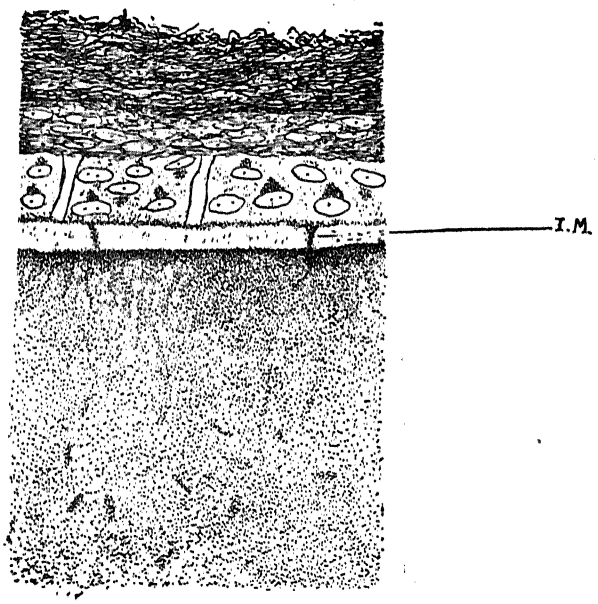

Fig. 2

to be only the beginning of a setback in this phenomenon. However, still the mitochondrial infiltration is quite prominent in all the birds investigated, although its occurence is generally in a haphazard manner, restricted to certain places. With the formation of the zona radiata the mitochondrial granules were observed to lie in it, establishing, so to say, connections at places with the follicular epithelium and the cortex of theoocyte (Fig. 2); so that in the clear background of the zona radiata may be seen at such places streaks of very fine granules of mitochondria making their way towards the periphery of the egg. This mitochondrial infiltration is, however, neither in the form of solid lumps nor so profuse as that of the Golgi bodies. As compared with the latter the mitochoodrial infiltration, in all the birds examined, remained throughout but a simple, less intensive, and, therefore, comparatively a much less impressive phenomenon liable in many cases to escape notice. Again, as in case of the Golgi bodies, once the infiltrated mitochondria managed to reach the cortex of the oocyte proper, it was impossible to differentiate between these and the similar organelle that originally belonged to the oocyte. Infiltration of mitochondria from the thecal layer to the follicular epithelium, as mentioned by Singh (1938) could not, however, be observed during the present investigation. But the way in which the mitochondrial granules were found to lie, specially their juxtanuclear position in the follicular epithelium, facing the thecal layer suggests very well the possibility of such an occurence.

Thus, the phenomenon of infiltration of the cytoplasmic organelles, from the egg membranes to the oocyte, has been observed in a fairly wide range of birds and the process is remarkably prominent.

DISCUSSIONS

To meet the Katabolic activities undertaken by the growing oocyte the nutrition of the egg demands an inflow of nourishing substance from every source, including through the intervening folds of the enveloping cells. As such as far back as 1870 Waldeyer thought of the passage of granules from the follicle cells to the oocyte. There have, however, been many—though only vague—attempts to explain the exact method by which the nutritive substances pass to the oocyte. Loyez (1905) finds that the function of the large follicle cells in reptiles is to furnish substances to the oocyte proper for the formation of yolk and she, therefore, suggests the passage of these sustances which may be fluid, semi-fluid or granular through canalicular prolorgations. This phenomenon of infiltrations has a definite bearing on the metabolic activities of the oocyte because the migrated Golgi bodies and mitochondria increase the resources of egg nutrition and help in the production of yolk. Thing (1918) opines that the increase in the size of the ovum is the consequent result of the accumulation of yolk by the follicle cells and that the nutritive substances are passed to the oocyte from the follicle cells.

The secretion of substances by the follicle cells into the eggs is, therefore, by no means a new idea although the cytological basis of this observance of the phenomenon of infiltration from the epithelium to the oocyte is indeed a later one mentioned to occur in such a definite manner originally by Bhattacharya (1925) in the tortoise *Testudo graeca* and Brambell in the fowl, *Gallus bankiva*, in the same year. Following this, the phenomenon has been confirmed by many a worker in both vertebrate and invertebrate animals.

This phenomenon of the infiltration of the Golgi bodies and mitochondria has

been rather sparingly recorded, particularly that of the latter inclusion. Brambell, as already referred to above, mentioned in *Gallus bankiva* only the transference of the golgi bodies from the epithelium to the egg but he was unable to throw any light as to the egg through the zona radiata. The exact process of the infiltration or passage of the Golgi apparatus from the follicular epithelium was shown for the first time by Bhattacharya in *Testudo graeca*. Later on, the same author in collaboration with others (1929, and 1937) was able to throw more light upon the problem by studying the phenomenon in birds and some other animals as well. The work of Ikeda (1928) in Japan further lent support to this phenomenon of infiltration by studying the problem in a number of birds. There are other workers as well who have described the Golgi infiltration from the follicle cells to the egg oocyte in various other animals alike as P. R. Bhattacharya (1929 and 1930) in mammals or Lal (1931 and 1934) in snakes or Clement (1933) in the squirrel. Among the invertebrates the infiltration phenomenon has been acceeded to by workers like Gardiner (1927) in *Limulus* who thinks that the Golgi elements in a way descend from the follicular epithelium. According to Steope (1926) also the Golgi bodies in *Nepa cinerea* increase in the egg partly by division and partly through the intrusion of the same from the follicle cells. Nussbaum-Hilarowcz (1917) observed the infiltration phenomenon of the mitochondria from the nurse-cells to the oocyte in *Dystiscus* while Govaerts (1913) reported the phenomenon in *Hymenoptera*.

Thus, it will be seen from these foregoing examples as well as what has been recorded during the course of the presents work, or a perusal of the literature bearing upon this phenomenon, that the infiltration theory of Brambell and Bhattacharya has been shown to exist in a large number of cases, although in some animals it may not occur in younger oocytes. For instance, Narain (1956) is unable to observe this infiltration of masses of Golgi bodies from the follicle cells in the younger oocytes of teleosts as was described by the original authors or as seen by the present author in the course of this investigation. De Robertis. Nowinski and Saez (1954) have also observed this phenomenon when they say, "The golgiosomes infiltrate into the interior of the ovocyte crossing the vitelline membrane".

The extensive work done from this aspect of the question leaves no doubt that the phenomenon of infiltration is strictly comparable in both vertebrate and invertebrate egg during growth and, therefore, the quantity so transferred depends on the actual need of the growing oocyte it may be more in such eggs which form huge quantities of yolk like birds and reptiles or less as in some of the fishes (Subramanium and Aiyer, 1936) or even rare in the higher mammals (Clement, 1933).

All the stages beginning with the accummulation of the Golgi bodies as well as mitochondrial granules at one pole of the cell and their passage through the zona radiata region when present were extensively seen.

The passage of mitochondria from the follicular epithelium into the oocyte itself in birds seems to be either not widely occuring a phenomenon or has hitherto escaped the notice of investigators, as compared with the similar phenomenon of Golgi bodies. It has been recorded only by three workers viz.. Ikeda (1928) in more than one bird, Das (1931) also in more than one bird and Singh (1938) in the Vulture. The present author has seen mitochondrial infiltration when the zona has not been established. Even after the formation of zona the infiltration seemed to continue. It should, however, be mentioned here that, with the full formation of the zona, the mitochondrial infiltration definitely decreased in intensity.

But this phenomenon of infiltration has been a common ground of controversy for those who refuse to consider the transference of these inclusions from the follicu-

lar epithelium to the oocyte as a case of infiltration. They suggest it as merely the accummulation of artefacts in that particular region. Jagersten (1935) is the chief amongst this group and question the validity of this infiltration phenomenon. Accordingly he questions the observation of Brambell (1925), Bhattacharya (1925) and Ikeda (1928) as he thinks it is by no means an easy task to determine merely by the study of sections on what side of the membrane a certain particle lies. Kirkman and Severinghaus (1938), while making a review of this phenomenon of infiltration of Golgi bodies, are also critical and observe thus: "It has been claimed (Bhattacharya 1925, 1929, 1930; Narain 1930, Bhattacharya and Lal 1929; Bhattacharya, Das and Dutt 1929; Narain 1930 and Lal 1933) that in certain inframammalian vertebrates the Golgi apparatus of the ovum and the oocyte receives a contribution from the cells of the follicular epithelium through a process of direct infiltration, but *verification* of such surprising observations is needed". (Italics mine). Some more work on the subject is needed to convince there two authors.

From this it is quite clear that this infiltration phenomenon has not only not attracted much of the attention of workers but also, as is natural, some of them are sceptical about it. From observations resulting from the present investigations the author recognises the phenomenon of infiltration because (i) the Golgi and mitochondrial elements present in the follicle cells are facing toward the egg-membrane presenting thus a *prima facie* case to support this contention, (ii) some of the Golgi elements are actually seen lying half-way between the follicle cells and the oocytes, (iii) the increase in the amount of the Golgi bodies and mitochondrial elements in the oocytes during its growth, as seen in the cortical band, at least partially, needs to be accounted for, and (iv) the fact that inspite of the general dispersal of these cytoplasmic organelles in the oocyte a peripheral band of the Golgi or mitochondrial elements is seen to continue to persist. In this contention of his the author is supported extensively by other workers as mentioned above the latest being Narain (1956).

Bhattacharya (1925) described a canalicular passage for the infiltration of Golgi bodies in the case of *Testudo graeca*. But during the course of the present investigation no such passage could be observed nor such a mention is made in birds by workers like Ikeda (1928), Das (1931), Srivastava (1934) and Singh (1938). It is, however, Das (1928) alone who has made a definite mention about the infiltration of Golgi bodies from the thecal cells to the epithelial layer while Singh (1938)—although not able to confirm the findings of Das (1931)—gives support to this part of the phenomenon only as far as mitchondrial infiltration is concerned. The present author is in agreement with the views expressed by these two authors that thecal infiltration is possible specially in view of the presence of the inclusions in the theca aggregated to face the epithelial layer; the reasons for the thecal infiltration also being to augument these organelles of the oocyte if and when these elements are meagre in quantity to meet the growing requirements of the oocyte itself.

Finally, the importance of this phenomenon is further enhanced, and it should prove of great significance, if we bear in mind the fact that the cytoplasmic inclusions have also been held responsible for the production of reserve food material of the oocyte. To all intents and purposes these maintain their usual quota of forming a concentrated layer at the cortex. Moreover, the final dispersal of these bodies as subsequent to the growth of the cytoplasmic expanse denotes the importance of these inclusions in the increasing metabolic activities of the oocyte.

SUMMARY

The ovaries of five species of Indian birds, *Accipiter nisus nisus*, *Holopterus ven-*

tralis, Ardeola grayii, Turdoides terricolor terricolor and *Acredotheres tristis tristis* were examined during various seasons in which only in case of *Ardeola grayii* striated zona radiata could be seen. Otherwise it continues to be a simple unstriated region.

The infiltration of the Gogi bodies from the epithelial cells into the oocyte was detected in all the birds examined. The extrusions of the elements from the follicular epithelium took place in granular form and not in lumps. After coming into the oocyte these enriched the peripheral concentration of the Golgi elements. No distinction, however, could be made out between those originally belonging to the oocyte, the Golgi bodies present in the follicular cells or those that had infiltrated.

The phenomenon of infiltration of mitchondrial element was also observed extensively during the present work. The infiltration of this element took place in a haphazard manner both before and after the formation of the zona radiata. The zona radiata remained a homogenous and non-striated structure in all the birds examined except in case of *Ardeola grayii* as said before. However, with the formation of the zona radiata the infiltration of mitochondria decreased only in intensity. Here again, as was the case in Golgi infiltration, no distinction could be made out between the mitochondrial elements that had infiltrated, those that accumulated at the periphery and those that were found in the follicular epithelium.

An additional source to augument in the agency for the elaboration of reserve food material by the oocyte is suggested to be the purpose of this infiltration phenomenon of the cytoplasmic organelles.

LETTERING AND EXPLANATION OF PLATE

D.C.	..	Dark staining cell
I. G.	..	Infiltration of Golgi material in patches
I. M.	..	Infiltration of mitochondrial material in patches
L. C.	..	Light staining cell
T. E.	..	Theca externa
T. I.	..	Theca interna
Z. R.	..	Zona radiata

Fig. 1 Part of an oocyte showing a portion of the theca, follicular epithelium and the periphery of the oocyte to show infiltration of Golgi bodies. Cajal toned and stained with Safranin and light green. 10×90 *Accipiter nisus nisus.*

Fig. 2 Part of an oocyte showing the infiltration of mitochondria haphazardly through zona radiata. F. W. A. with Champy-Kull staining. 10×90 *Acridotheres tristis tristis.*

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MONOGENEA OF INDIAN FRESH-WATER FISHES*

V. *Dactylogyrus multispiralis* n. sp. (SUBFAMILY DACTYLOGYRINAE) FROM THE GILL FILAMENTS OF *Silondia silondia* (Ham.), FROM LUCKNOW, INDIA.

By

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(Read at the Twenty-sixth annual Session of the Academy at Aligarh on 4th February, 1957)

While working on the monogenetic trematodes infesting the fresh-water fishes commonly found in Lucknow, the present author came across an interesting species of *Dactylogyrus* which forms the subject of the present paper. Several specimens of this trematode were recovered from the gill filaments of *Silondia silondia* (Ham.). The recovery of trematodes was made by the refrigeration technique of Mizelle (1936). Measurements of all chitinised structures were made after the method suggested by Mizelle (1938).

DACTYLOGYRUS MULTISPIRALIS n. sp.

Elongated dactylogyrids (Fig. 1), body length 0·62-0·78 mm., width at cephalic lobes 0·034 mm., maximum body width 0·086 mm. Head organs, four pairs. Eye-spots, two pairs, posterior larger than the anterior pair. Pharynx 0·037×0·032 mm., intestine bifurcate, crura confluent posteriorly.

Haptor (Fig. 2) 0·08-0.083 mm. in length, 0·058-0·074 mm. in width. Armature of the haptor consists of a pair of anchors, a bar, a pair of short supporting bars and seven pairs of hooks. Anchors with expanded superficial and insignificant deep root, each with single wing not reaching upto the points. Length of the anchor 0·043-0·049 mm., width of its base 0.01 mm., length of superficial root 0·01-0·011 mm. Bar straight, with anteriorly directed extremities, 0·023-0·026 mm. in length. Supporting bars lie at the base of the anchors, each 0·01-0·012 mm. Hooks sickle-shaped, consisting of a base, a shaft, a sickle-shaped portion and an apposable piece. Length of hooks 0·006-0·008 mm.

Testis intercaecal, 0.02-0.03×0·1-0·13 mm. Vas deferens slender, vesicula seminalis not clear. Copulatory complex (Fig. 3) consisting of a very long spirally coiled cirrus and an accessory piece. Base of the cirrus slightly swollen, the number of cirrhal loops varies from 15 to 19, the diameter of the loops being 0·049-0·076 mm. The accessory piece is firmly articulated to the distal end of the cirrus, consisting of

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* Being part of the thesis approved for the Ph. D. degree of Lucknow University, 1955.

5-6 plates arranged in a floral fashion. Ovary pyriform and pretesticular, 0·045-0·06X 0·02-0.027 mm. Vagina (Fig. 4) sinistral, chitinised and tubular, with flask-shaped mouth continued into a coiled vaginal tube ending into the receptaculum seminis. There are two transverse and three circular loops in the vaginal tube. Vitellaria from pharynx to much beyond the caecal union. Vitelline ducts slender. Single egg (Fig. 5), observed in some specimens, oval with a blunt projection at the narrower end, measures 0·04 × 0·06mm.

DISCUSSION

The present form is assigned to the genus *Dactylogyrus* Diesing, 1850, on account of the presence of a pair of anchors and a bar in the haptor. Since the creation of another genus *Neodactylogyrus* Price, 1938, the genus *Dactylogyrus* has been most discussed by a number of authors. *Neodactylogyrus* has been recognised by Price (1938), Kimpel (1939), Sproston (1946) and Yin and Sproston (1948), rejected by Mizelle and Donahue (1944), Monaco and Mizelle (1955), and ignored by Gussev (1953), Mizelle and Klucka (1953) and Mizelle and Webb (1953). The present writer recognises the validity of *Neodactylogyrus*, restricting the species with one bar to the genus *Dactylogyrus* and those with two bars to the genus *Neodactylogyrus*.

Of all the known species of the genus *Dactylogyrus*, the present form shows affinities with D. *apogonis* Yamaguti, 1940, D. *auriculatus* (Nordmann, 1832) Diesing, 1850 and D. *cordus* Nybelin, 1937. From all these species, however, the present form can be clearly distinguished in the characteristic shape of the cirrus which is thrown into definite loops, numbering 15-19, and the nature of the accessory piece, consist-ing of 5-6 plates arranged in a floral fashion at the distal end of the cirrus.

Further, from D. *apogonis* the present form can be clearly distinguished in (1) the shape of the anchor bases, (2) comparative small size of the bar, and (3) very small size of the hooks. From D. *auriculatus* and D. *cordus* the present form can be distinguished in (1) possession of conspicuous supporting bars in the haptor, (2) the shape of the bar, (3) the shape of the hooks.

The present from is, therefore, assigned to a new species of *Dactylogyrus*, which is named **D.** *multispiralis*, the specific name indicating the nature of the cirrus. It will be interesting to note here that this is the second species of this genus from India, the first was described by Price (1938) from two fresh-water fishes of Mysore.

ACKNOWLEDGMENT

The present studies were carried out in the Department of Zoology, Lucknow University, Lucknow, under the guidance of Professor M. B. Lal. The writer is indebted to him for the valuable criticizm and constant encouragement throughout the course of the work. Acknowledgments are also due to the Government of India for the award of a Research Training Scholarship to carry out the present work.

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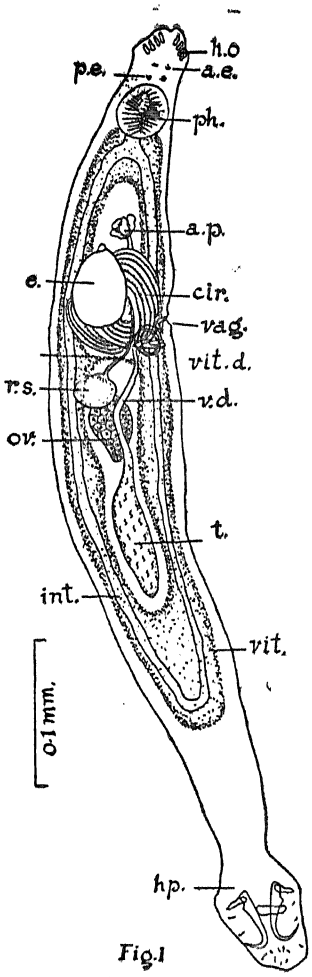

Fig.1

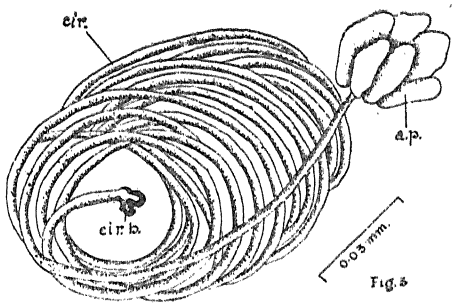

Fig.3

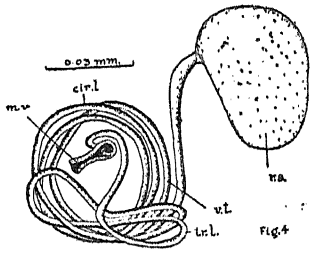

Fig.4

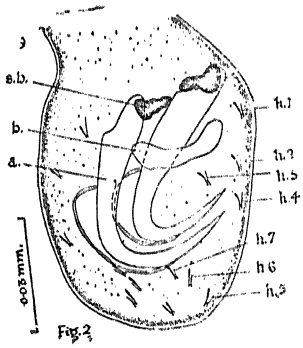

Fig.2

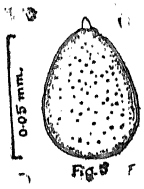

Fig.5

EXPLANATION OF FIGURES

Fig. 1. *Dactylogyrus multispiralis* n. sp. Entire worm, dorsal view. a.e.-anterior eye-spots, a.p. -accessory piece, cir.-cirrus, e.-egg, h.o.-head organs, hp.-haptor, int.-intestine, ov.-ovary, p.e.-posterior eye-spots, ph.-pharynx, r.s.-receptaculum seminis, t. testis, vag.-vagina, v.d.-vas deferens, vit.-vitellaria, vit.d.-vitelline duct.

Fig. 2. *Dactylogyrus multispiralis* n. sp. Haptor. a.-anchor, b.-bar, h.-1-h.7.-hooks 1 to 7 s.b.-supporting bar.

Fig. 3. *Dactylogyrus multispiralis* n.sp. Copulatory complex. a.p.-accessory piece, cir.-cirrus, cir.b.-base of cirrus.

Fig. 4. *Dactylogyrus multispiralis* n.sp. Vagina. cir.l .-circular loop, m.v.-mouth of vangina, r.s.-receptaculum seminis, tr. l.-transverse loop, v.t.-vrgi al tube.

Fig. 5. *Dactylogyrus multispiralis* n.sp. Egg.

ON THE STRUCTURE OF THE GOLGI BODIES IN THE NERVE CELLS OF FUNAMBULUS PALMARUM LINN., THE INDIAN PALM SQUIRREL

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ABSTRACT

The study of Golgi bodies in the nerve cells of various animals has resulted in diverse observations and interpretations. Further, there is a controversy about the homologous nature of the Golgi bodies and the sudanophile bodies. The present investigations, on the neurones and spinal ganglion cells, point out that the Golgi bodies and sudanophile bodies occur both in localized and distributed conditions. Not only they are closely related topographically but they also show one constant structural plan namely wrapping of osmiophile, argentophile and sudanophile material, partially or completely, over non-neutral stainable vacuoles. Evidences have been pointed out and discussed in support of the homologous nature of the Golgi bodies and sudanophile bodies in the present communication.

INTRODUCTION

The reticular pattern of the Golgi apparatus, advocated by various cytologists (Golgi, Beams, Gatenby & Moussa) has been the most disputed topic even upto the present day. The study of these bodies in nerve cells, resulting in diverse interpretations and observations, has cleavaged the cytologists into opposed groups-some supporting the reticular pattern, others opposing it, still others advancing new patterns.

Golgi (1889) discovered the reticular apparatus placed in juxtanuclear position in the spinal ganglion cells of cattle and horse embryos and in the latter the apparatus was found displaced somewhat towards the periphery of the cell. Esterman and Gitlitz (1927), however, failed to observe the Golgi apparatus in adult cats by the silver methods, although they observed the same in younger animals.

Later Holmgren's discovery of *trophospongium* as a system of clear spaces or tubes in the nerve cells, and its homology with the Golgi apparatus, produced some confusion. Cajal (1908, 1915) accepted this homology but Golgi and Penfield (1921) did not subscribe to Holmgren's views.

Penfield (1921) showed that the Golgi apparatus and trophospogium were distinctly

separate inclusions as the Golgi apparatus underwent a series of changes after axon section; while the Holmgren's canal remained uneffected; the two elements also differed in form and arrangement. On the other hand Beams (1931) maintained that the Golgi apparatus and Holmgren's trophospongium are homologous structures but differed from Holmgren by pointing out that the Golgi apparatus is entirely cytoplasmic and never reaches the surface. Legendre's (1910) homology of the apparatus with Nissl substances was also refuted by Beams and Penfield who demonstrated the two elements simultaneously.

Parat (1924) opposed the reticular pattern of the Golgi apparatus and on his observations propounded the well known Parat's vacuome theory, neutral red stainable vacuoles constituting the Golgi apparatus. Although subsequent work by Covell and Scott (1928), Baker (1944), Gatenby and Moussa (1950) have elucidated and established the morphological basis of the Golgi apparatus, the conceptional interpretation of the structures still present us with radically conflicting views. My studies on Golgi apparatus (1956) led me to re-examine the validity of these different views, especially due to the constant and complete picture of these structures obtained by me in the neurones and in the ganglion cells of the spinal cord.

MATERIAL AND METHODS

Thin slices of the spinal cord of the squirrel, cut transversely, were fixed in the living condition and treated by the following methods :—

(1) Ludford's modification of Mann-Kopsch (vide Lee's Vade-mecum).
(2) Silver methods of Da Fano and Aoyama.
(3) Baker's (1944) Sudan black method for the Golgi bodies in frozen sections.

Spinal ganglion cells were also fixed and processed by the methods mentioned above. Vital staining with neutral red as recommended by Baker (1949) was also carried out both in the neurones and the spinal ganglion cells but the results were not satisfactory.

Paraffin sections were cut at (5μ) and they were bleached and toned respectively after methods (1) and (2) while frozen sections were cut at (15μ) after method (3). Silver and osmium methods were strictly controlled to avoid any distortion of the picture of Golgi apparatus. In addition to this few mitochondrial preparations, fixed by shridde method and stained by Iron-alum-haematoxylin, were prepared and studied. Osmicated and silvered sections were studied under ordinary microscopy as well as phase miscroscopy. The study under phase miscroscopy revealed a greater structural detail of the pattern of the Golgi apparatus, particularly in the material fixed after Ludford's modification of Mann-Kopsch, as is particularly apparent if we compare Figs. 3 & 4 of plate 1. Fig. 3 which is a phase picture of the spinal ganglion cells reveals clearly the vacuolar component of the Golgi apparatus which is, however, not visible in the same section (Fig. 4) with ordinary microscopy. Such a useful comparison was initiated by Barer (1947) who first advocated the study of fixed material as well with phase contrast miscroscopy.

OBSERVATIONS

In osmium tetroxide preparations of neurones the Golgi apparatus has been observed in the localized condition as well as in the distributed one, and consists of a system of vacuoles around each of which osmicated condensed substances make a compact wrapping. The phase contrast microphotograph (Fig. 5) clearly demons-

trates the vacuolar component of the apparatus. The spinal ganglion cells show both the localized as well as the distributed conditions of the Golgi apparatus (Plate 1; Figs. 1, 2, 3 & 4). The localized Golgi bodies have been clearly observed at two places, one on each side of the nucleus (Fig. 3). In all the cells studied, a constant structural plan of the apparatus is obtainable, namely argentophile or osmiophile substances wrapping partially or completely the vacuolar component. This picture is always constant although the cells may show different orientations of the Golgi bodies. The vacuoles are not neutral stainable vacuoles. Even in those spinal ganglion cells (Plate 1; Fig. 1) which show distributed condition of the Golgi bodies more or less like a perinuclear ring around the nucleus, the osmiophile substances are observed as filaments or rings partially or completely covering the vacuoles respectively. At one point in Fig. 1 (Plate 1) the vacuole (V) is seen surrounded clearly like a ring by the osmiophile component (Os. C). Similarly in Figs. 3 & 5 the wrappings of osmiophile condensed substances over the vacuoles are clearly distinguishable.

In sudan black preparations of the neurones (Plate 2, Fig. 7 & 8), made according to Baker's method, the same structural plan of the sudanophile bodies as that of the Golgi bodies is revealed. In such preparations the sudanophile substances are observed as filaments or rings encircling partly or completely the vacuoles; in addition to sudanophile grains being embedded on the cortex of the vacuoles (Plate 2, Fig. 7 A). Thus it is clear that there is a close structural similarity between these inclusions. Further, there is a topographical relationship between the sudanophile bodies and the Golgi bodies, in as much as that both are found in the distributed as well as in the concentrated conditions (compare Fig. 7 & 8 with Fig. 1 & 5 respectively). Invariably in all these cases, localization of Golgi bodies or sudanophile bodies was observed more in young nerve cells, while in contrast the old ones showed the distributed phase.

It is thus clear that in all my preparations the picture of the Golgi bodies is constant, but it presents two phases, one localized and the other scattered or distributed.

DISCUSSION

Covell and Scott (1928) claimed that the classical Golgi network of the nerve cells is an artefact produced by the fusion of neutral red stainable vacuoles and granules during fixation; and that on these the excessive precipitation of osmium or silver takes place. On the other hand Beams (1931) showed in spinal ganglion cells of the rat that Golgi bodies and neutral red stainable granules are separate inclusions i.e they are not homologous. Later on Baker (1944) advanced the view that the Golgi apparatus consists of rows or groups of neutral red stainable vacuoles; and that around such vacuoles the lipid substance formed cortices, the reticular pattern of the apparatus being artificially produced by running together of these lipid cortices and forming strings or mesh-works over which reduction of silver nitrate or osmium tetroxide takes place. Thomas (1948), working on the sympathetic neurones of mouse and rabbit, also showed the Golgi apparatus to consist of a dispersed system of spherical bodies, each made of a neutral red staining core (the vacuome of Parat) enveloped in a lipodal sheath or cortex. In 1949 Baker no longer held the vacuoles to be neutral red stainable and thus his original conception of the four Golgi components became reduced to two, as the specificity of the netural red to the vacuoles and also the presence of diffused lipoidal substances between the vacuoles were excluded from his first plan of the Golgi body components.

So we see that recently doubts have been cast on the specificity of neutral red in revealing the Golgi apparatus. Gatenby and Moussa (1950) are of the opinion that the neutral red staining is not known to reveal the Golgi apparatus in any vertebrate cell. The present observations lend a strong support to the later modified view of Baker (1949) for in my preparations the neutral red does not stain the Golgi bodies, and also there is no sign of diffused lipoidal substance after sudanophile preparations. Further no separate neutral red stainable bodies seem to exist as reported by Beams (1931) in rat spinal ganglion cells. In view of these observations made by Baker and now by me, it is difficult to support Moussa's (1952) argument based on Beams (1937) and his (Moussa) collaborator-Gatenby's observations that since neutral red is non-specific for Golgi bodies, the vacuoles themselves are non-existent. His argument wants to disprove the association of vacuoles in the Golgi bodies of Baker's conception, inspite of the fact that Baker himself no longer believes in the specificity of neutral red to the vacuolar component. Moussa may be justified in not believing in the "neutral red Golgi apparatus" of Parat, Covell and Scott or Thomas, which is also supported by the present observations but certainly his attempt to reject Baker's modified conception (1949) stands on flimsy grounds. It will, therefore, be seen that there are few cytologists who believe in the original Parat's vacuome hypothesis but certainly the modifications introduced by Baker have found a great deal of support from various workers including myself. In the present observations both in the case of neurones and spinal ganglion cells the location of condensed osmicated substances, covering either partially or completely the vacuolar component, is more or less similar to the Baker's plan.

It may be stated that simultaneous occurrence of localized Golgi apparatus and discrete Golgi elements both in the spinal ganglion cells and in the neurones have never been observed to occur in the present investigations. The presence of either the localized or the scattered Golgi bodies in these nerve cells, unfolds the possible interchangeability of the two conditions during different phases of nerve cell activity. Kwan (1936), studying the spinal ganglion cells of the rabbit, has also pointed out the evolutionary changes in the Golgi apparatus during embryonic development. According to him, in the initial stages the Golgi bodies appear as numerous black granules, whereas in advanced stages they are gathered at one pole of the nucleus, later on forming a reticulum. At birth the rabbit shows the reticulum completely surrounding the nucleus. Similar observations have been noted by Alexenko (1920) in the spinal cord of chick.

In the squirrel the localized condition can be explained by aggregation of Golgi elements in the juxtanuclear position, due to restriction of activity in the neighbourhood of the nucleus in young nerve cells. This may well be compared to the state of affairs of the Golgi bodies in developing oocytes. In the oocytes we find that the Golgi bodies are concentrated at one pole of the nucleus (Tewari 1956). In older cells, with the spreading of cytoplasmic activity, it is possible that the Golgi elements from the concentrated Golgi system seggregate to other parts of the cytoplasm resulting in discrete scattered Golgi elements. That is why we do not find the concentrated and the scattered conditions occuring simultaneously in the same cell.

My view, therefore, is that it is simply a matter of aggregation and seggregation of Golgi elements which produce the localized and scattered condition, and that these two conditions, are different phases of the same elements caused by localized or spread-out activity of the Golgi bodies. This is also a first report of the fact that localization may take place at one or more places (Fig. 3, Plate 1) in the spiral ganglion cells.

Moussa (1952), working on neurones of mouse, has unconsciously supported the present interpretations, by holding that "The Golgi apparatus in young, half grown and adult mice, forms a compact network around the nuclear membrane; in old animals it fragments to a greater or lesser degree and its distribution becomes more wider". The "fragmentation" of Golgi apparatus, as suggested by Moussa, can be replaced by the more appropriate word "seggregation", since the structure appears as a compact network due to excessive precipitation of osmium or silver over the aggregated vacuoles.

Some of the workers such as Hirsch (1939) and Thomas (1948) have brought in the role of mitochondria in the production of Golgi complex. Hirsch believes that the Golgi apparatus originates from mitochondria as "*Praesubstanz*" which stains with Janus green as well as with neutral red. Thomas also has coined a new term "*Mitochondrial Golgi complex*" which is produced by the running together of sudanophile bodies and filamentous mitochondria. Adamstone (1952), working on spinal ganglion of young pigs has also observed close association of mitochondria on the surface of the reticulum of Golgi apparatus. The present observations do not support the possible production of Golgi apparatus as a result of association of mitochondria. Mitochondrial preparations of the present meterial have shown the granular type of mitochondria with no fixed orientation, as well as by the conspicuous absence of the vacuolar elements. Therefore, from the present studies it will not be justified to assign a mitochondrial role in the production of Golgi apparatus.

Further, it will not be out of place to discuss here the views of many cytologists who believe in reticular nature of the Golgi apparatus, although slightly modified from the original conception, in as much as they have shown the reticulum not as a solid structure but of canalicular nature.

Beams (1931) in spinal ganglion cells of rat has observed the Golgi apparatus in the form of a typical paranuclear network, as an incomplete reticulum, or as isolated filaments of osmiophilie substance.

As mentioned previously Adamstone (1952) in spinal ganglion cells of young pigs has described the Golgi apparatus as a canalicular cytoplasmic reticulum with close association of mitochondria on the surface of net. More recently Barer (1947) in phase pictures of unstained spinal cord of monkey has shown white canalicular system of the Golgi bodies. Moussa (1952) in sympathetic neurones of mouse described the Golgi apparatus in the form of a more or less complete network as perinuclear canals. In neurones these canals are very narrow, enveloped by thick argentophile Golgi substance, while in spinal ganglion cells these canals are comparatively very wide. The present observations do not support any of the above conditions as in these cells a uniform pattern consisting of vacuoles invested by osmiophile substances is forthcoming. The canals, as shown by Moussa, are untraceable in all the preparations and, therefore, it is difficult to reconcile to Moussa's view who holds that,"The canalicular nature of the Golgi apparatus is well shown when the Golgi filament is transversely sectioned or when it is cut longitudinally through the canal.....Some parts of the Golgi apparatus do not show canals, in this case the section is passing through the wall parallel to the canal". The mere fact that there are no signs of variations from one constant pattern of the apparatus in the spinal ganglion cells of squirrel, even when sections are cut in different places, is sufficient to disprove Moussa's view.

Lately the sudanophilie nature of the osmiophile substance of the Golgi apparatus as suggested by Baker has aroused much controversy. Gatenby & Moussa (1949,

1950) have classified the sudanophile material in mammalian neurones as follows "(a) free sudanophile granules and sometimes rings and crescents distributed at random in the cytoplasm. They were first described as lipid bodies by Ciaccio in 1910, and then redescribed as the true "Golgi elements" by Baker in 1944. Thomas in 1948 refers to these bodies as spheroid complexes or mulberry spheroids or Golgi complement or Baker's bodies. These bodies are too few and small to form the relatively very large Golgi apparatus of vertebrate neurones. Nevertheless a more or less intimate connection exists between some of these sudanophile bodies and the Golgi substance. Furthermore these bodies are minute and few (sometimes absent) in young animals whereas the true Golgi apparatus is at the height of its development. In old animals the reverse is the case, i.e. the sudanophile bodies greatly increase in number and size whereas the Golgi apparatus is at its minium size; (b) sudanophile scales or leaf like bodies spread partly around the individual parts of argentophile Golgi substance. These are very clear and numerous in the spinal ganglion, very few and difficult to make out in the spinal cord cells and usually lacking in sympathetic neurone; (c) pigment granules which are characteristic of old neurones..... The pigment granules have been mistaken by Baker and Thomas for the Golgi apparatus".

On the contrary in the present observations, neurones treated by Baker's sudan black method show scattered sudanophile bodies (Figs. 7, 7A; Pl. 2) and somewhat concentrated sudanophile bodies (Fig. 8; Pl. 2) more or less similar to Golgi body's plan illustrated by Figs. 1, 2, 6 (Pl. 1) and Fig. 5 (Pl. 1) respectively. In all the sudanophile preparations the association of vacuoles with lipoidal substances is a constant feature similar to the associations of vacuolar components with osmiophile substances in Golgi bodies preparations.

From these observations, therefore, two things are clear i. e., that the Golgi bodies and sudanophile bodies both occur in localized and scattered conditions and further basic pattern of these inclusions in both these conditions is one and the same, namely, constant association of vacuoles with osmicated (=lipoidal) investing substances.

Therefore the same topography of discrete Golgi elements and scattered sudanopile bodies and that of concentrated Golgi bodies and sudanophile bodies together with the same background of structural plan go to prove Baker's conception that the sudanophile bodies and the Golgi bodies are one and the same thing. At the same time these observations give a strong reply to views of Moussa (1952) who differentiates the Golgi bodies and the sudanophile bodies simply by localized condition of the former and the distributed one of the latter. The question of differentiation of these two inclusions merely on the orientiation ground does not arise at all as the identical conditions are available in both the cases of the Golgi bodies and the sudanophile bodies. In the present material the Golgi bodies themselves do not bear any fixed orientation , particularly in the spinal ganglion cells, which show clearly the localized Golgi apparatus at one or two places and also discrete Golgi elements distributed at random in the cytoplasm. So will it be justified to say, in the light of Moussa's arguments, that these two types of the Golgi bodies are different ? The structural plan of argentophile and osmiophile as well as the sudanophile preparations have never shown the duplex pattern of the Golgi apparatus or the sudanophile bodies.

As mentioned previously in young nerve cells compact Golgi bodies (=sudanophile bodies) are observed due to concentration of activity near about the nucleus but in older cells, due to distribution of activity to other parts of the cell, seggregation of the Golgi elements (sudanophile bodies) takes place and, therefore, it is but natural

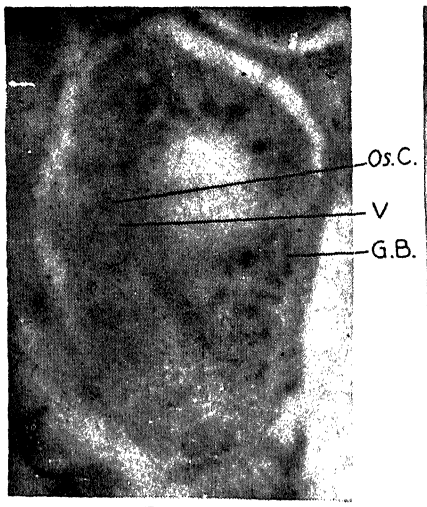

FIG. 1.

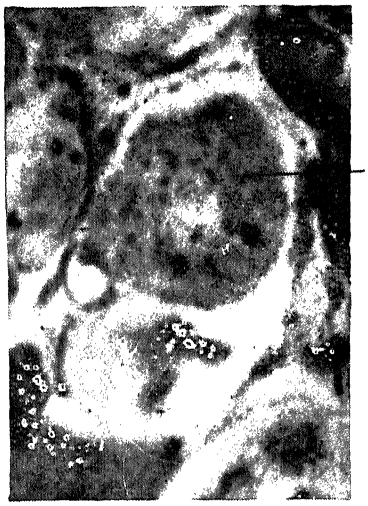

FIG. 2.

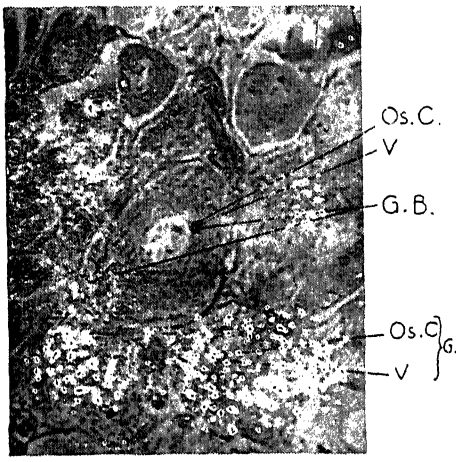

FIG. 3.

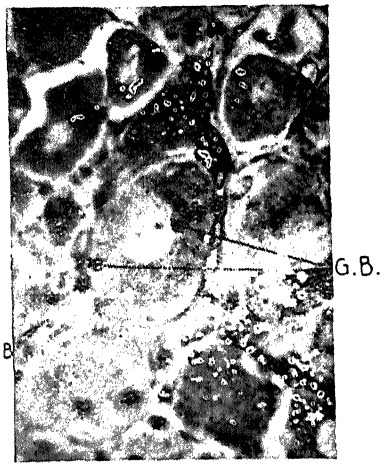

FIG. 4.

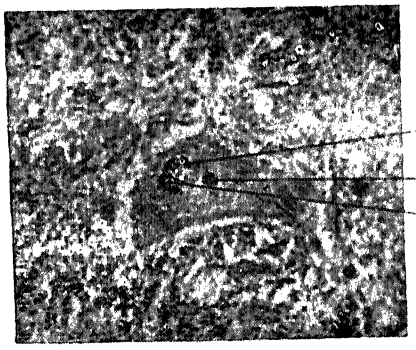

FIG. 5.

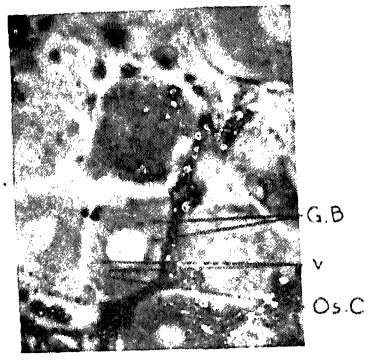

FIG. 6

PLATE 1

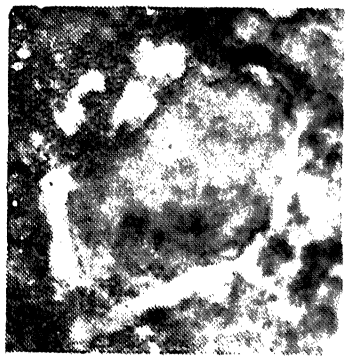

FIG. 7.

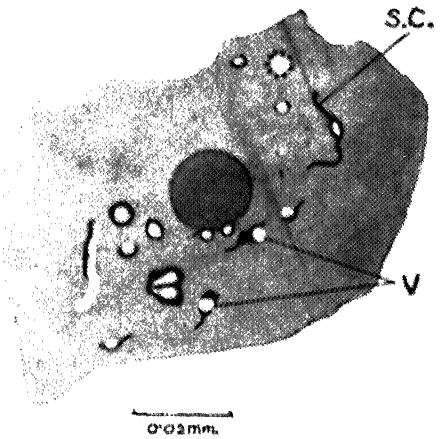

FIG. 7A.

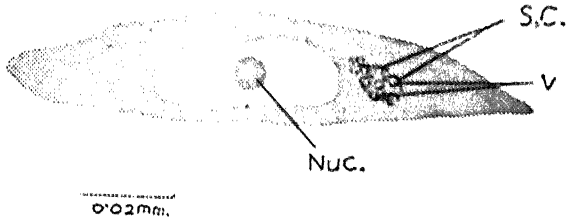

FIG. 8.

PLATE. 2.

to foresee that in old cells the seggregated elements will be observed and not the concentrated system and in young cells the concentrated system will be observed and not the seggregated elements.

So are these arguments not sufficient to dislodge Gatenby and Moussa's apprehensions in the above quoted lines who hold that, "Further more these bodies are minute and few (sometimes absent) in young animals whereas the true Golgi apparatus is at the height of its development. In old animals, the reverse is the case i.e. the sudanophile scales or leaf like bodies greatly increase in number and size where the Golgi apparatus is at its minimum size".

It will be further clear from the present observations that neither the scattered Golgi elements nor the scattered sudanophile bodies are so small in size or number so as not to constitute the localized conditions (compare Figs. 3 &8 with Figs. 1 & 7 respectively).

As a matter of fact each Golgi body is associated with a vacuolar component, similar to the sudanophile bodies, and so if scattered elements (either Golgi bodies or sudanophile bodies) become aggregated or concentrated on one side or on two sides of a nucleus due to restriction of activity in the neighbourhood of the nucleus in young nerve cells, we may get the compact localized conditions (compare Figs. 1 & 2 with Fig. 3 and Fig. 7 with Fig. 8). It is in this stage of concentrated system of Golgi bodies in young nerve cells that the excessive precipitation of osmium or silver produces the artificial net-like structure of the Golgi apparatus, so often reported by various workers.

Therefore it is concluded that the argentophile, osmiophile and sudanophile substances represent the trimorphic nature of one and the same condensed substance, covering either partially or completely the other vacuolar component and the two constituents together represent the Golgi apparatus. Further, it seems reasonable to deduce that the Golgi bodies and the sudanophile bodies are homologous structures on the evidences of their similar structural plans and orientations.

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EXPLANATION OF PLATES

Plate 1. (Microphotographs)

Fig. 1. A spinal ganglion cell showing vacuolar and osmicated components of the scattered Golgi bodies, somewhat distributed in perinuclear position. Ludford. × 1770.

Fig 2 A spinal ganglion cell showing distributed condition of the Golgi bodies Ludford. × 1387.

Fig. 3. Spinal ganglion cells showing the localized Golgi bodies with the osmicated and vacuolar components. Ludford. Phase contrast. × 470.

Fig. 4. Spinal ganglion cells of fig. 3 photographed with ordinary microscopy with the same magnification (× 470). Note that the vacuolar and osmicated components are not clearly distinguishable.

Fig. 5. Neurone showing the juxtanuclear localized Golgi apparatus with osmicated and vacuolar components. Phase contrast. × 572.

Fig. 6. A group of spinal ganglion cells showing partial or complete wrapping of osmicated components over the vacuolar part in the distributed Golgi elements. Ludford. × 482.

Plate 2:

(A microphotograph and camera lucida drawings).

Fig. 7. Neurone showing the scattered sudanophile bodies, Baker's sudan black method (for frozen section).

Fig. 7A. Camera lucida drawing of fig. 7 showing the structural details of the sudanophile bodies, illustrating the close association of sudanophile bodies and the vacuoles.

Fig. 8. Neurone showing juxtanuclear concentration of sudanophile bodies. Baker's sudan black method (for frozen section).

LETTERING OF THE FIGURES

G. B.—Golgi bodies;
Nuc—Nucleolus; Os. C. — Osmicated component;
S. C.—Sudanophile Component; V—Vacuoles.

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* Not consulted in originals.

STUDIES ON THE NUTRITION OF FUNGI

1. THE INFLUENCE OF DIFFERENT SOURCES OF CARBON ON THE GROWTH AND SPORULATION OF *Colletotrichum capsici* (SYD.) BUTLER & BISBY

By

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INTRODUCTION

The importance of the studies on the nutrition of fungi is exceedingly great in order to understand their behaviour fully. Vegetative growth, growth habits, and reproduction (both asexual and sexual) are markedly influenced by the type of nutrients supplied. The study of fungal nutrition has great practical importance when applied to fungi such as yeasts, penicillia, aspergillii etc. The knowledge of nutrition of fungi has enabled us to use some of them as test organisms for vitamins, trace elements etc. in various analytical procedures. Such a study is also important in systematic mycology since the structure and development of fungi are most easily studied on pure culture on reproducible media (Hawker, 1950). The controlled variation of the composition of suitable artificial media has enabled the study of fungal metabolism. As pointed out by Nickerson and Williams (1937), the knowledge of the nutrition of pathogenic fungi may throw light on the cause of their parasitic mode of life and further progress may thus be made toward the specific treatment or prevention of the diseases caused by them.

Very extensive and critical studies on the nutrition of fungi are well under way in other countries (Nickerson & Hopkins, 1947; Wolf and Wolf, 1947; Hawker, 1950; Lilly & Barnett, 1951). In recent years several papers have appeared on the nutrition of fungi in India, some of which are mentioned under discussion. It is aimed to carry on such studies in this laboratory as well. This first paper is a preliminary report on the influence of different carbon sources on the growth and sporulation of *Colletotrichum capsici* (Syd.) Butler & Bisby, the incitant of the die-back of chillies (*Capsicum annum Linn.*)

MATERIAL AND METHOD

Several monosporic isolates of *C. capsici* were made from the infected fruits and stems of chillies, collected from different localities of Amritsar and Gurdaspur districts (Punjab). Monosporic isolate CC 5, which showed rapid rate of growth and profuse sporulation, was selected for investigating the carbon nutrition of the fungus, and is deposited in the Herbarium of the Punjab University. The basal medium C (Dextrose 20 gm., KNO_3 5gm., KH_2PO_4 1 gm., $MgSO_4$ $7H_2O$ 0·5 gm., $Fe_2(SO_4)_3$ $6H_2O$ 0·005 gm. and Distilled Water 1000 ml.), devised by the authors, was found to be quite good for

the growth of the fungus and was employed throughout the present work. Unless otherwise stated 50 ml. of the culture media were pippetted into 250 ml. Erlermeyer flasks and sterilized at 15 lbs. pressure for 15 minutes. Initial pH of the media was adjusted to 6 by the colorimetric method, with the addition of dilute KOH or HCl solution, as earlier trial experiments showed it to be the optimum for the growth of the fungus. Buffering of the media was not necessary because pH of the basal medium was shown to remain within the optimum range for growth of the fungus throughout the present studies.

Stock cultures of the fungus were maintained on potato dextrose agar (peeled and sliced potatoes 200 gm., dextrose 20 gm., agar agar 17 gm., and Distilled water 1000 ml.) slants by repeated subculturing at an interval of two weeks. This medium was found quite suitable for growth and sporulation of the present organism.

Spore suspension was prepared in sterile distilled water with the inoculum obtained from seven days old colonies grown on potato dextrose agar. Seeding of culture flasks was done by adding 1 ml. of the same spore suspension to each flask with the help of sterilized 1 ml. pippettes.

The glass-ware was always cleaned with potassium dichromate sulphuric acid solution and rinsed at first thoroughly with tap water and finally with distilled water. Analytical grade chemicals (C. P. or Analar) were mostly employed for the various experiments.

All experiments were conducted in stationary cultures which were incubated at 32°C (with a variation of 1°C) for ten days, as preliminary work had shown that maximum growth of the fungus was reached under these conditions. Ultimately the cultures were filtered through previously weighed and dried Watsman filter papers, dried to a constant weight in a hot-air-oven at 70° C, and weighed with an analytical balance after allowing to cool in a desiccator. Special attention was devoted to the process of filtration to avoid the error in determination of dry weight caused by the phenomenon of sopping referred to by Bretzloff (1954) in the case of *Sordaria fimicola*. The data collected on dry weight of the mycelium and presented in the following experiments, represent the mean determination of atleast three replicates. Macroscopic observations on sporulation were also recorded and the data are represented by the following signs based on visual observations :—

—	No sporulation.
+.....	Poor sporulation.
++	Fair sporulation.
+++	Good sporulation.
++++	Excellent sporulation.

EXPERIMENTAL WORK

Thirty-nine carbon compounds comprising 18 carbohydrates, 10 organic acids, 6 alcohols and 5 miscellaneous ones were tested as sole sources of carbon for mycelial growth and sporulation of *C. capsici*. The basal medium C (excluding dextrose) was taken and sterilized at 15 lb. pressure for 15 minutes. The various carbon compounds were dissolved separately in distilled water and the pH was adjusted to the neutral point to minimize the possibility of hydrolysis during autoclaving. Sterilization of carbon compounds was done by autoclaving at 10 lbs. pressure for 10 minutes only. The compounds were then added separately and aseptically to the

remainder of the medium. *Each carbon compound was added at a concentration calculated to provided 400 mgs. of carbon per 50 ml. of the basal medium, which amount of carbon is contained in 20 gm. of dextrose present in one litre of the basal medium. The media in the flasks were seeded and incubated at 32° C for 10 days after which period the data on dry weight of the mycelium, shift in pH, and sporulation were recorded. Ten days were found to be sufficient for recording the growth because maximum weight was reached by that time. The complete data on carbon nutrition which are summarized in tables 1 & 2 were collected in three sets of experiments conducted under indentical conditions.

EXPERIMENTAL RESULTS

The data on carbohydrates as sole sources of carbon are summed up in table 1 and it is indicated that *C. capsici* is capable of utilizi ng nearly all the carbohydrates, with of course varied growth response on each individual carbohydrate. Pentose sugars like d(—) arabinose, ribose and some other carbohydrates like lactose and inulin yielded only poor mycelial growth. Fairly good growth occured on 1(+) arabinose, cellobiose, melibiose, raffinose, pectin and sorbose while good growth was observed on fructose, mannose, melezitose, and starch. Maximum growth, however, occured on dextrose, sucrose and maltose. Growth was markedly different on 1(+) and d(—) forms of arabinose, being much more on 1(+) form than on d(—) form.

The type of carbohydrate exercised a profound influence on sporulation of the fungus. The carbohydrates which supported most luxuriant mycelial growth were not necessarily most favourable for sporulation. No sporulation was observed on sorbose although it supported fairly good mycelial growth; instead only sclerotial formation was stimulated by this hexose sugar. Sporulation on d(—) arabinose was also absent. Fairly good sporulation occurred on 1 (+) arabinose, ribose, dextrose, lactose, melibiose and raffinose. Fructose, mannose, sucrose and melezitose yielded good sporulation while excellent sporulation occured on pectin, maltose and starch.

TABLE 1

Growth and sporluation of C. capsici in media containing various carbohydrates as sole sources of carbon after I0 days incubation at 32° C. Initial pH adjusted to 6

Carbon source	Mean dry weight of mycelium in mgs./50 ml. of medium.	Sporulation grade	Final pH
Ribose	37	++	6·8
1 (+) arabinose	134	++	7·1
d (—) arabinose	13	—	6·5
Dextrose	278	++	6·4
Fructose	234	+++	6·9
Sorbose	109	S[1]	7·0
Mannose	244	+++	7·0

*Starch, inulin and pectin were added at the rate of 18 gms. each per liter of the basal medium.

Galactose[2]	—	+	7·2
Sucrose	271	+++	6·5
Lactose	69	++	6·8
Cellobiose	133	++	5·8
Maltose	268	+ + + +	7·3
Melibiose	178	+ +	5·6
Melezitose	207	+ + +	7·4
Raffinose	160	+ +	6·5
Starch[2]	--	+ + + +	7·5
Inulin	41	+	7·0
Pectin	179	+ + + +	7·8

The shift in pH of the media containing various carbohydrates was not much marked.

Organic acids, alcohols and other carbon compounds

The data summarized in Table 2 indicate that various organic acids and alcohols, and oils are only poor substitutes of sucrose in the carbon nutrition of the fungus. Tartaric acid which supports fairly good mycelial growth, however, is an exception to this general remark. No growth occured on formic acid, acetic acid, propionic acid and glycolic acid and isopropyl alcohol. Growth on oxalic acid, stearic acid, methyl alcohol, ethyl alcohol, butyl alcohol, dulcitol, mannitol, acetone, acetamide, xylol, castor oil and olive oil was negligible, while that on succinic acid, lactic acid, tartaric acid and citric acid was somewhat better.

Sporulation was entirely lacking in a majority of organic acids, alcohols, and other carbon sources tested. Poor sporulation occurred on succinic acid, ethyl alcohol, butyl alcohol and mannitol but that on lactic acid was fairly good. Tartaric acid, in strorg contrast to other organic acids, evoked excellent sporulation as was observed with pectin and maltose.

The reaction of the culture media containing organic acids like succinic acid, lactic acid, tartaric acid, and citric acid tends to shift towards the alkaline side. The change in pH with other organic acids, alcohols or oils etc. is not well marked.

DISCUSSION

Brock (1951) remarked that while interpreting the data on growth in relation to the supply of different carbon compounds, it is pertinent to bear in mind that growth is a very complex physiological process and as such its magnitude is never determined by a single nutritional factor, but on the other hand a set of interdependent environmental factors are always involved. Judged under this context, the present work on *C. capsici* has a limited scope in view of the fact that only the source of carbon was varied while other factors were kept uniform. Some useful information is however, still available when it is borne in mind that potassium nitrate was the only source of nitrogen throughout the experiments on carbon nutrition.

S[1] No sporulation but only sclerotia were developed.
2. Dry weight not determined due to difficulties in separating the mycelium from the culture medium.

TABLE 2

Growth and sporulation grade of C. capsici in media containing miscellaneous carbon compounds as sole sources of carbon, after 10 days incubation at 32° C. Initial pH adjusted to 6.

Carbon source	Mean dry weight of mycelium in mgs./50 ml. of medium.	Sporulation grade	Final pH
Sucrose (control)	304	+ + +	6·5
Formic acid	0	—	6·2
Acetic acid	0	—	6·3
Propionic acid	0	—	6·2
Stearic acid	15	—	6·3
Oxalic acid	5	—	5·5
Succinic acid	77	±	7·3
Glycolic acid	0	—	5·5
Lactic acid	51	+ +	8·1
Tartaric acid	157	± + + +	9·2
Citric acid	59	—	8·1
Methyl alcohol[1]	7	—	6·3
Ethyl alcohol[1]	29	+	6·3
Isopropyl alcohol[1]	0	—	6·3
Butyl alcohol[1]	17	+	6·2
Dulcitol	14	—	6·4
Mannitol	11	+	6·5
Acetone	16	—	6·2
Acetamide	5	—	6·3
Xylol	7	—	6·5
Castor oil[2]	—	—	6·9
Olive oil[2]	—	—	7·0

A majority of the carbohydrates tested supported very good mycelial growth of the fungus. Carbohydrates like d-arabinose, ribose, sorbose, lactose and inulin proved to be only poor substitutes of sucrose while others like l-arabinose, cellobiose, melibiose, raffinose and pectin were fairly good substitutes of sucrose. Good mycelial growth occured on fructose, mannose, melezitose and starch. Best growth, however, was observed on dextrose, sucrose and maltose. *C. capsici* has shown considerable measure of agreement with *Colletotrichum indicum* (Ramakrishnan 1947) in its carbon requirements. Lactose, for example is a poor source of carbon for both these fungi whereas sucrose and maltose have been reported to yield maximum growth of *C. indicum*, as has also been observed for *C. capsici*. Tandon and Aggarwal

1. Added without sterilization.

2. Dry weight not recorded because of difficulties in separating the mycelium from the medium.

(1956) reported excellent growth of 3 species of *Gloeosporium* with mannitol. However, *C. capsici* showed very poor growth on mannitol. *C. capsici* also differs from these three species with respect to its growth on some other carbon compounds. *C. capsici* shows similar response to various carbon compounds as did various species of *Pythium* (Saksena and Mehrotra, 1949). Neither of these authors studied the effect of optical activity of arabinose or other carbon compound on the growth of their fungi. Mathur et al (1950) reported galactose as the best and starch as poor sources of carbon for the sporulation of *Colletotrichum lindemuthianum* but reverse was the situation with *C. capsici* which showed best sporulation with starch and poor sporulation with galactose. In its ability to utilize pectin, *C. capsici* shows resemblance with *Venturia inaequalis* (Leben & Keitt, 1948). The differential growth response observed on l(+) and d(—) forms of arabinose can apparently be explained in terms of optical activity of this pentose sugar. Somewhat similar data on l(+) and d(—) forms of arabinose was reported by Steinberg (1942) on *Aspergillus niger* and Brock (1951) on *Morchella esculenta*. While mannose is a good source of carbon, its corresponding alcohol mannitol is very poor for the vegetative growth of *C. capsici*, which indicates that structural difference between the carbon compounds, is a factor involved in determining the extent of growth, as was shown by Steinberg (1942) and Cartino (1949).

Organic acids, in general, have been found to be poor sources of carbon for the growth of *C. capsici*. A critical examination of the data on organic acids, presented in Table 2, reveals that the number of carboxylic groups in an organic acid has a bearing on its utilization by the fungus. Thus rganic acids like formic acid, acetic acid, propionic acid and glycolic acid which possess only one carboxylic group support no growth of the fungus while others like succinic acid, lactic acid and citric acid having more than one carboxylic group, are much better sources of carbon and support relatively fair growth of the fungus. Much more growth on tartaric acid than on citric acid seems to be due to the possession of two hydroxyl groups in the former than one such group in the latter. It seems that both the number of hydroxyl and carboxylic groups in an organic acid affects its utilization by *C. capsici*. The view of Hawker (1950) that only the number of hydroxyl groups affects the utilization of an organic acid, as a source of carbon shall have to be modified in the light of the present work.

Sporulation was markedly influenced by the source of carbon employed and the response observed was not equally favourable in all cases. Compounds supporting good vegetative growth were not necessarily equally good for sporulation. Best sporulation was observed on maltose, starch and pectin and this is in agreement with the statement of Hawker (1950) that complex carbohydrates like disachharides and polysachharides are more favourable for sporulation of fungi in general.

SUMMARY

Monosporic isolates of *C. capsici* (Syd.) Butler & Bisby were made from the infected fruits and stems of chillies (*Capsicum annum* Linn.) and a suitable isolate CC5 was selected for further work on carbon nutrition. Studies on the nutrition of the pathogen were carried out in liquid basal medium C in stationary cultures. Preliminary work showed that the fungus, when incubated at 32° C for ten days on the basal medium C with initial pH adjusted to 6, yielded good growth.

The growth responses of the fungus to different carbon compounds as sole sources of carbon were studied. Good mycelial growth and sporulation did not necessa-

rily occur on the same carbon source. Best mycelial growth occurred on dextrose, sucrose, and maltose. Good growth occured on fructose, mannose and melezitose. Fairly good growth occured on 1(+) arabinose, cellobiose, melibiose, tartaric acid, raffinose and pectin. Poor growth occured on d(—) arabinose, sorbose, lactose, inulin, a majority of organic acids, various alcohols and oils. No growth occurred on formic acid, acetic acid, propionic acid, glycolic acid and isopropyl alcohol.

Best sporulation occured on pectin, maltose, starch and tartaric acid. Good sporulation occured on fructose, mannose, sucrose and melezitose. Fairly good sporulation occured on 1(+) arabinose, ribose, dextrose, lactose, cellobiose, melibiose, raffinose, alanine, glutamic acid and lactic acid. Poor sporulation occured on galactose, inulin, succinic acid, ethyl alcohol, butyl alcohol, mannitol. In general complex carbohydrates like disachharides and polysachharides proved to be more favourable for sporulation than for mycelial growth.

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STUDIES ON THE NUTRITION OF FUNGI

II. THE INFLUENCE OF DIFFERENT SOURCES OF NITROGEN ON THE GROWTH AND SPORULATION OF *Colletotrichum capsici* (SYD.) BUTLER and BISBY

By

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(Received on 22nd December 1957).

It has been shown in the first paper in the series (Thind and Randhawa 1957) that growth and sporulation of *Colletotrichum capsici* are markedly influenced by the various carbon compounds. This paper deals with the influence of different nitrogenous compounds on the growth and sporulation of the same pathogen.

MATERIAL AND METHODS

The material and methods were the same as already mentioned in the first paper on the series. The same basal medium C (dextrose 20 gms., KNO_3 5 gm., KH_2PO_4 1 gm., $MgSO_4.7H_20$ 0.5 gm., Fe_2 $(SO_4)_3.6H_20$ 0.005 gm. and distilled water 1,000 ml.) was employed throughout the present work on nitrogen nutrition.

EXPERIMENTAL WORK

Thirty-two nitrogenous compounds comprising 8 inorganic, 18 amino acids and 6 miscellaneous organic compounds were tested as sole sources of nitrogen for the growth and sporulation of *C. capsici*. The various nitrogenous compounds* were added to the basal medium C (excluding potassium nitrate) in amounts calculated to furnish 693 mgs. of nitrogen per litre, which amount is contained in 5 gm. of potassium nitrate present in one litre of the basal medium C. The nitrogen compounds were autoclaved separately at 10 lbs. pressure for 12 minutes and then added separately and aseptically to the remainder of the autoclaved medium. Initial pH of the media was adjusted to 6. The media in the flasks were seeded with the same spore suspension and incubated at 32°C for 10 days. The complete data on dry weight of mycelium, sporulation, and shift in pH which were collected in two separate sets of experiments conducted under identical conditions, are given in Table 1 and 2.

TABLE 1

Growth and sporulation of *C. capsici* in media containing different inorganic and or-

*Peptone, yeast extract, and casein were added in amounts equal to that of Potassium nitrate.

ganic nitrogenous compounds as sole sources of nitrogen after 10 days incubation at 32° C. Initial pH adjusted to 6.

Nitrogen source	Mean dry weight of mycelium in mgs./50 ml. of medium	Sporulation*	Final pH
Control	0	0	6.0
Potassium nitrate	352	+ +	6.5
Sodium nitrate	336	+ + +	6.6
Potassium nitrite	0	0	6.2
Sodium nitrite	0	0	6.0
Ammonium chloride	104	+	4.1
Ammonium sulphate	145	+	5.0
Ammonium nitrate	279	+ +	5.4
Ammonium phosphate	264	+ +	4.8
Urea	434	+ + + +	7.0
Peptone	240	+ +	7.4
Yeast extract	415	+ + + +	6.2
Casein	68	+	4.0
Asparagin	119	+ + +	7.0
L-glutamine	419	+ + + +	7.2

EXPERIMENTAL RESULTS

Inorganic Nitrogenous compounds :—

The data summarized in Table I show that *C. capsici* is capable of utilizing both the nitrate and ammonium nitrogen but growth on nitrites of sodium and potassium is completely inhibited. The nitrate nitrogen is decidedly far better than ammonium nitrogen and furthermore ammonium phosphate and ammonium nitrate support quite good growth in contrast to ammonium chloride or ammonium sulphate. No growth occurred in the complete absence of nitrogenous compounds.

Sporulation varied considerably on different sources of nitrogen. Good sporulation occurred on sodium nitrate; fairly good on ammonium nitrate, ammonium phosphate and potassium nitrate; and very poor on ammonium chloride or ammonium sulphate.

Organic Nitrogenous Compounds :— (Table 1)

While some of the organic nitrogenous sources like urea, glutamine and yeast extract served as excellent sources of nitrogen for the mycelial growth, others such as peptone displayed good growth and still others like asparagin and casein proved to be

*The rating of sporulation into four classes represented by plus sings was based on carefui visual observation only.

quite inferior. Excellent sporulation occurred on urea, glutamine and yeast extract; good on asparagin and peptone but poor on casein. On the whole organic nitrogen proved to be much more favourable than the inorganic nitrogen for the growth and sporulation of *C. capsici.*

Amino acids :—

The data presented in Table 2 indicate that several of the amino acids tested served as excellent source of nitrogen for the mycelial growth of *C. capsici.* Best growth was observed on L-leucine, dl-leucine, arginine, alanine, valine, proline, glutamic acid, dl-isoleucine, glycine and aspartic acid; fairly good on phenyl alanine, tryptophane and histidine; and poor on threonine, tyrosine, cystine , methionine and lysine.

Excellent sporulation was observed on l-leucine, dl-leucine, proline and arginine; good on alanine, valine, isoleucine and glutamic acid; fairly good on cystine; and poor on glycine, tryptophane, aspartic acid, lysine and histidine. No sporulation occurred on threonine and tyrosine, the latter alone induced only sclerotial development. Phenyl alanine, although supported fairly good growth, did not yield any sporulation.

TABLE 2

Growth and sporulation of *C. capsici* in media containing different amino acids as sole sources of nitrogen after 10 days incubation at 32° C. Initial pH adjusted to 6.

Nitrogen source	Mean dry weight of mycelium in mgs/50 ml. of medium.	Sporulation grade	Final pH
Control	0	0	6.0
Glycine	484	+	7.0
Dl-alanine	600	+ + +	4.2
Dl-valine	596	+ + +	7.0
L-leucine	637	+ + + +	4.8
Dl-leucine	617	+ + + +	6.2
Dl-isoleucine	554	+ + +	4.4
Dl-threonine	12	0	6.5
Dl-b-phenylalanine	372	0	3.9
L-tyrosine	171	S1	4.0
L-tryptophane	299	+	3.6
L-proline	591	+ + + +	5.2
L-cystine	147	+ +	4.0
Dl-methionine	102	+	4.3
Dl-aspartic acid	463	+	7.5
L-glutamic acid	566	+ + +	6.8
Dl-lysine dihydrochloride	128	+	4.5
L-arginine monohydrochloride	601	+ + + +	4.0
L-histidine monohydrochloride	378	+	4.1

1 No sporulation but only sclerotia werd formed.

DISCUSSION

C. capsici has shown considerable nutritional versatility by its ability to utilize diverse types of nitrogenous compounds. The ability of the fungus to utilize nitrate nitrogen, ammonium nitrogen, and organic nitrogen and furthermore its failure to utilize atmospheric nitrogen justifies its inclusion in group II of Robbin's classification of Fungi and Bacteria based on their nitrogen requirements (Robbins 1937). Nitrate nitrogen supported much more growth of the fungus than ammonium nitrogen. Less growth with ammonium compounds can be partly attributed to the shift in pH towards the acidic side in media containing these salts. The observation that ammonium phosphate supported quite good growth, in contrast to ammonium chloride or ammonium sulphate, lends support to the view held by Hawker (1950) that ammonium phosphate is a better source of nitrogen than ammonium chloride or ammonium sulphate because inhibiting hydrogen ion concentration is reached less rapidly with this salt as the sole source of nitrogen than with the other two. Nitrites of sodium and potassium inhibited the growth of the fungus, as has been shown by several other investigators for other fungi (Ramakrishnan, 1941; Gordon, 1950; Patel et al 1950, Srivastava, 1951, Tandon and Grewal, 1956). That nitrites exerted a toxic effect on growth is quite logical because the pH at which the fungus was cultured lies on the acidic side where nitrites are known to be toxic. At acid pH values nitrites are usually in the form of undissociated nitrous acid which thus produces the unfavourable effect on growth (Cochrane and Conn, 1950 ; Nord and Mull, 1945.).

A majority of amino acids proved to be favourable sources of nitrogen for growth of the fungus. Methionine, cystine tyrosine, and lysine were found to be poor sources, as was reported with some other fungi by Steinberg (1942) and Leben and Keitt (1948). Tandon and Grewal (1956), however, found good growth of *Gloeosporium musarum*, *G. papayae* and *Colletotrichum papayae* on methionine. Pelletier and Keitt (1954) made a loose classification of amino acids into "good" (alanine, arginine, aspartic acid glutamic acid, glycine and proline), "fair" (histidine, isoleucine, serine, tyrosine, and valine), and "poor" (cystine, hydroxyproline, leucine, lysine, methionine, phenylalanine, threonine and tryptophane) nitrogen sources on the basis of their utilization by a large number of diverse fungi. The amino acids tested as sole sources of nitrogen for the growth of *C. capsici* can also be similarly classified as follows : "good" sources of nitrogen: leucine, alanine, arginine, glycine, aspartic acid, valine, proline, glutamic acid and isoleucine; "fair": phenylalanine, tryptophane, and histidine, "poor": threonine, tyrosine, cystine, methionine and lysine. It becomes quite apparent from the above that *C. capsici* shows general agreement with other fungi in its pattern on amino acid utilization.

Among the organic nitrogenous compounds tested, urea and gultamine served as excellent sources of nitrogen for mycelial growth of *C. capsici*. Brock (1951) and Gordon (1950) also obtained similar results in their fungi. Tandon and Grewal (1956) also found urea to be a good source of nitrogen for their three fungi. Urea, however, yielded poor growth of the fungi investigated by Srivastava (1951). Peptone is successfully worked upon by *C. capsici* and yielded a fairly good growth. Lockwood et al (1936) and Srivastava (1951) also found similar results with peptone on *Rhizopus oryzae* and *Alternaria tenuis* respectively, while Tandon and Grewal (1956) found significantly good growth of their fungi with peptone.

The source of nitrogen exercised a profound effect on sporulation of the fungus. In general compounds which supported best mycelial growth, yielded excellent spo-

rulation; conversely compounds supporting poor growth yielded poor sporulation. Another significant observation worth pointing out is that excellent sporulation was always yielded by organic nitrogenous compounds which are thus obviously more suitable than inorganic nitrogenous compounds for sporulation of *C. capsici.* Inorganic nitrogenous compounds, peptone, casein, glycine, threonine, phenylalanine, tyrosine, tryptophane, cystine, methionine, aspartic acid, lysine and histidine yielded fair, poor to no sporulation of *C. capsici*. Tandon and Grewal, 1956 also got fair, poor to no sporulation of their fungi with glycine, phenylalanine, methionine, aspartic acid but good sporulation with nitrates, peptones, and histidine. *C. capsici* yielded good or excellent sporulation with urea, yeast extract, aspargine, glutamine, alanine, valine, leucine, isoleucine, proline, glutamic acid and arginine as was also found by Steinberg (1942) with his fungus *Aspergillus niger.* Tandon and Grewal, 1956 found that *Gloeosporium papayae* failed to sporulate on glycine, leucine and glutamic acid but these compounds yielded fair sporulation with *G. musarum* and *Colletotrichum papayae.*

SUMMARY

Studies on the nitrogen nutrition of *Colletotrichum capsici* were carried out in the liquid basal medium C and data on dry weight and sporulation was recorded after 10 days incubation at 32° C. The fungus was able to utilize nitrates, ammonium nitrogen, organic nitrogen but not the atmospheric nitrogen. Nitrites completely inhibited the growth. Nitrate nitrogen proved to be far better than ammonium nitrogen for sporulation as well as mycelial growth. Organic nitrogen, in general, was much better utilized than inorganic nitrogen. Maximum mycelial growth occurred on leucine, alanine and arginine whereas excellent sporulation occurred on leucine, proline, arginine, urea and glutamine. Good growth occurred on valine, proline, glutamic acid, urea, glutamine, yeast extract, potassium nitrate, sodium nitrate glycine and aspartic acid while good sporulation occurred on alanine, valine, glutamic acid, sodium nitrate, yeast extract and asparagin. Fairly good growth occurred on ammonium nitrate, ammonium phosphate, peptone, phenylalanine, tryptophane and histidine while fairly good sporulation occurred on cystine, potassium nitrate, ammonium nitrate, ammonium phosphate, and peptone. Poor to no growth and poor to no sporulation occurred on the rest of the nitrogenous compounds.

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MONOGENEA OF INDIAN FRESH-WATER FISHES

VI. THREE NEW TREMATODES BELONGING TO THE GENUS *Neodactylogyrus* PRICE, 1938 (FAMILY DACTYLOGYRIDAE), FROM SOME FRESHWATER FISHES OF LUCKNOW, INDIA.

By

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Gyrodactyloidea has been mainly reported from the fresh-water fishes of North America, although a few records are also available from Russia, Europe, Australia and Japan. From India, however, the records have been astonishingly few. With a view to extend our knowledge in this group of parasites, the present studies were carried out in the Department of Zoology, Lucknow University during the years 1952-55.

Our knowledge of the family Dactylogyridae from Indian fresh-water fishes is limited to five trematodes (Price, 1938; Thapar, 1948; Jain 1952; Jain, 1952a and Jain, 1955). The genus *Neodactylogyrus* Price, 1938 is still unrepresented from India. In the present paper I have described three new species of this trematode.

MATERIALS AND METHODS

Fishes for the present investigation were purchased from the Lucknow Fish Market, at Kaiserbagh. The identification of hosts were made from the papers of Shaw and Shabbeare (1937) and Chauhan and Ramakrishan (1953).

Due to large amount of mucus present in the gill filaments of fishes, the trematodes do not separate off easily and any mechanical means to do so results in damage to the parasites. To overcome this difficulty Mizelle's (1936, 1938) refrigeration technique was employed. Freezing the fishes had the simultaneous effects of killing the parasites in a relaxed condition and of breaking up the mucus, both of which facilitate examination and preservation. Another method employed for the removal of parasites was Hargis' (1952, 1953) Chloretone technique. The latter method has additional advantage of saving time and labour. For the study of structures of critical taxonomic importance, such as the armature of the haptor, copulatory complex etc., trematodes were studied in glycerine mounts. In measuring curved surfaces, Mizelle's (1936, '38) method was employed. Measurements were made across the line subtending the greatest arcs described by those structures i.e., in the case of anchors from the proximal tip of the longest root to the distal end of the curve.

Neodactylogyrus calbasi n.sp.

Host : *Labeo calbasu* (Ham.)
Location : Gill filaments.
Locality : Lucknow, India.
Host specimens examined : seven.
Host specimens infected : five.
Number of parasites studied : Ten.

Elongated dactylogyrids (Fig. 1), body length 0.58-0.84 mm., body width 0.12 mm., width at cephalic lobes 0.05-0.06 mm. Two pairs of cephalic lobes with three pairs of head organs. Eye-spots in two pairs. Pharynx oval, 0.035 × 0.03 mm Pharyngeal glands well differentiated, made up of small globular bodies, on eithe side of the pharynx. Intestine bifurcate, crura confluent posteriorly.

Haptor (Fig.2) is somewhat discoidal, 0.07-0.12 mm. in length and 0.085-0.15mm. in width. Peduncle stout, 0.065-0.09 mm. in width. Armature of haptor (Fig. 3) consists of a pair of anchors, a pair of bars and seven pairs of hooks. Each anchor has a stout base, a strong shaft and fine recurved points. The superficial root is blunt while the deep root is represented by a number of indentations. Each anchor with double wings, extremely reduced, not reaching upto the points. Length of the anchor 0.048-0.067 mm., width of the base 0.031-0.034 mm. Ventral bar with rounded and downwardly projecting extremities, 0.06-0.063 mm. in length. Dorsal bar stout, Y -shaped with three pointed projections in the mid-third of its body. Length of the dorsal bar 0.042-0.046 mm., width at the anterior arms 0.046-0.049 mm., width in the region of middle projections 0.025-0.027 mm. Seven pairs of hooks, similar in shape but dissimilar in size, hook pairs 1-5 are marginal and ventral, hook pairs 6 and 7 median and dorsal. Each hook sickle-shaped, consisting of an elongated stout base, a thin shaft, a stout sickle-shaped portion and a curved apposable piece. Dimensions of hooks as follows: I pair 0.027-0.033 mm., II pair 0.032-0.038 mm., III pair 0.026-0.027 mm., IV pair 0.044-0.045 mm., V pair 0.012-0.013 mm., VI pair 0.04-0.041 mm., VII pair 0.041-0.042 mm.

Testis intercaecal and somewhat spindle-shaped, 0.09-0.12× 0.02-0.031 mm. Vesicula seminalis, short spindle-shaped, situated near copulatory complex. Copulatory complex (Fig. 4) consisting of cirrus and accessory piece, firmly articulated at the base. Cirrus tubular, thick-walled, measuring 0.078-0.081 mm. In many specimens it is seen to project a little outside the body of the trematode. Accessory piece, with swollen base and extremity having claw-like projections, measuring 0.075-0.078 mm. Ovary ovoid, pretesticular, 0.051-0.063×0.02-0.027 mm. Vagina, sinistral, chitinised with a horse-shoe shaped body at mouth leading by a transverse vaginal tube to the receptaculum seminis. Single egg (Fig. 5), observed in some specimens, thin-shelled, oval with a blunt projection at the narrower end, measuring 0.054×0.028 mm. Vitellaria from pharynx to the union of the intestinal crura.

Discussion. Of all the known species of the genus *Neodactylogyrus* Price, 1938, the present form comes closer to *N. affinis* (Bychowsky, 1933) Price, 1938 *N. kulwieci* (Bychowsky, 1933) Price, 1938 and *N. haplogonus* (Bychowsky, 1933) Price, 1938. It can, however, be clearly distinguished from these species in several important respects.

From *N. affinis* and *N. kulwieci*, the present form can be distinguished in (1) nature of the cirrus which is firmly articulated with the accessory piece at the base, (2) the shape of the accessory piece, (3) the shape of the anchor bases, and (4) conspicuous differential development of the hooks.

From *N. haplogonus*, the present form can be distinguished in (1) the shape of the dorsal bar, (2) the shape of the anchors and (3) the nature of the hooks.

It is evident, therefore, that the present form is different from all the known

species of *Neodactylogyrus*. It is designated to a new species which is named *N. clabasi*, after the host.

Neodactylogyrus indicus n.sp.

Host: *Puntius stigma* Cuv. & Val.
Location: Gill filaments.
Locality: Lucknow, India.
Host specimens examined: sixteen.
Host specimens infected: two
Number of parasites examined: Seven.

Slender, elongated dactylogyrids (Fig. 6), body length 0.95-1.35 mm., maximum body width 0.12-0.15 mm., width at cephalic lobes 0.12-0.13 mm. Two pairs of cephalic lobes bearing three pairs of head organs. Head organs of each side joined by a common duct. Eye-spots in two pairs, anterior and posterior, the latter with larger number of melanistic granules. Pharynx, oval, 0.055×0.03 mm. Pharyngeal glands, taking bright stain with Haematoxylin, on both sides of the pharynx. Intestine bifurcate, crura confluent posteriorly. Outlines of intestine, in many specimens, obscured by the dense vitellaria spread in that region.

Haptor (Fig. 7) octagonal to somewhat discoidal, 0.11-0.135 mm. in length, 0.091-0.12 mm. in width. Peduncle 0.072 mm. wide. Armature of haptor (Fig. 8) consists of a pair of anchors, a pair of bars and seven pairs of hooks. Anchors with stout base, short shaft and fine recurved points. Anchor bases with long superficial roots while the deep root is represented by two indentations. Wings on anchors reduced and do not reach upto the points. Length of anchor 0.058-0.065 mm., width of the base 0.016-0.017 mm., length of the superficial root 0.018-0.02 mm. Ventral bar long straight shaft, 0.045-0.051 mm. in length. Dorsal bar stout and characteristic Y-shaped with a pair of arms in the middle. Length of the dorsal bar 0.043-0.048 mm., width at the anterior arms 0.016-0.033, mm., width in the region of the middle arms 0.028-0.03 mm. Seven pairs of hooks, similar in shape subequal in size. Each hook sickle-shaped, with elongated base, an insignificant shaft, a sickle-shaped portion and a curved apposable piece. The dimensions of hooks are as follows: I pair 0.034-0.039 mm., II pair 0.028-0.039 mm., III pair 0.03-0.032 mm., IV pair 0.03-0.034 mm., V pair 0.031-0.036 mm., VI pair 0.032-0.038 mm., VII pair 0.036-0.038 mm.

Testis slender, elongted, 0.3-0.36×0.01-0.02 mm. Vas deferens long and ending at the base of the copulatory complex. Vesicula seminalis not differentiated. Copulatory complex (Fig. 9) consisting of a stout cirrus, a small accessory piece and a long coiled cirrhal thread. Cirrus stout, tubular with somewhat triangular base and swollen terminal end. It measures 0.043-0.047 mm. Accessory piece weakly developed, being platelike in shape, 0.013-0.015 mm. in length. The cirrhal thread is about four times the length of the cirrus. Ovary rounded and pretesticular, 0.04-0.046 ×0.02-0.026 mm. Vagina sinistral, vaginal tube compactly coiled (Fig.10), opening into spindle-shaped receptaculum seminis. Vitellaria from pharynx to the caecal union. Vitelline ducts slender, joining near the receptaculum seminis. No egg has been observed in the specimens studied.

Discussion. Of all the known species of the genus *Neodactylogyrus* Price, 1938, the present form shows affinities with *N. affinis* (Bychowsky, 1933) Price, 1938, *N. kulwieci* (Bychowsky, 1933) Price, 1938 and N. *calbasi* n. sp. It can, however, be clearly distinguished from these species in important respects.

From N. *affinis* and *N. kulwieci*, the present form can be distinguished in (1) the shape of anchor bases, (2) poor development of accessory piece, (3) sickle-shaped nature of the hooks and (4) presence of a cirrhal thread in the copulatory complex.

From *N. calbasi* n. sp. the present form can be distinguished in (1) shape of ventral bar, (2) the size of the fifth hook which is almost similar in size to the remaining hooks, (3) the shape of the accessory piece, and (4) presence of cirrhal thread.

It is evident, therefore, that the present form is different from all the known species of the genus *Neodactylogyrus* Price, 1938. It is here designated to a new species which is named N. *indicus*.

Neodactylogyrus cotius n. ps.

Host : *Rohtee cotio* (Bloch).
Location : Gill filaments.
Locality : Lucknow, India.
Host specimens examined : eleven.
Hot specimens infected : three.
Number of parasites studied : eight.

Elongated dactylogyrids (Fig. 11), body length 0.65-0.74 mm., body width 0.1 mm., width at the cephalic lobes 0.04-0.06 mm. Two pairs of cephalic lobes, (Fig. 12) with five pairs of head organs. Eye-spots in two pairs, the anterior and the posterior pair, the latter with a larger number of melanistic granules. Pharynx oval, 0.02-0.029x 0.025-0.031 mm. The outlines of the intestine could not be worked out due to large amount of vitellaria spread in that region.

Haptor (Fig. 13) discoidal, 0.052-0.067 mm., in length and 0.05-0.065 mm. in width. Peduncle short and stout, 0.04 mm. in width. Armature of haptor (Fig 14) consists of a pair of anchors, a pair of bars and seven pairs of hooks. Each anchor with a stout base, a short shaft and fine recurved points. Anchor bases bifurate, with long blunt superficial and short blunt deep root. Wings on anchors not clear. Length of anchor 0.024-0.027 mm., width of base 0.015-0.017 mm. Ventral bar is stout transverse shaft, slightly bent in the middle, with anteriorly directed extremities, measures 0.021-0.023 mm. in length. Dorsal bar slender with rounded extremities and measures 0.019-0.021 mm. in length. Of the seven pairs of hooks, 1-5 are arranged marginally on the ventral surface while 6 and 7 are median and dorsal in position. Each hook is crochet-shaped, consisting of an elongated base, a shaft and a crochet-shaped portion. The dimensions of the hooks are as follows ; I pair 0.01-0.012 mm., II pair 0.016-0.019mm., III pair 0.017-0.018 mm., IV pair 0.013-0.018 mm., V pair 0.015-0.018 mm., VI pair 0.008-0.009 mm., VII pair 0.008-0.009 mm.

Testis slender, elongated, 0.18-0.21 × 0.01-0.02 mm. Vesicula seminalis not clear. Copulatory complex (Figh. 15) consists of a long coiled cirrus and a spoon-like accessory piece, both non-articulate at the base. The base of cirrus is swollen and the tubular portion is thrown into three coils. The maximum diameter of each coil is as follows : I coil 0.01-0.012 mm., II coil 0.013-0.014 mm., III coil 0.024-0.025 mm. The accessory piece is made up of a ring and a curved handle, its total length being 0.027 mm., and maximum diameter of ring is 0.01 mm. Ovary, ovoid and

pretesticular, 0.045×0.025 mm. Vagina not clear. No egg has been observed in the specimens studied.

Discussion. The present form shows affinities with *N. simplicimalleatus* (Bychowsky, 1931) Price, 1938, *N. wunderi* (Bychowsky, 1931) Price, 1938, *N. bulbus* (Mueller, 1938) Price, 1938, *N. orchis* (Mueller, 1938) Price, 1938, *N. confuscus* (Mueller, 1938) Price 1938, *N. acus* (Mueller, 1938) Price, 1938 and *N. rubellus* (Mueller, 1938) Price, 1938, from amongst all the known species of the genus *Neodactylogyrus* Price, 1938. It can , however, be clearly distinguished from each of these species in important re-respects.

From *N. simplicimalleatus*, the present form can be distinguished in (1) the three-looped nature of the cirrus, (2) the shape of the accessory piece, (3) the shape of the dorsal bar, and (4) the nature of the hooks.

From *N. wunderi*, the present form can be distinguished in (1) the three-looped nature of the cirrus, (2) the shape of the accessory piece, (3) the shape of the anchor bases, and (4) the nature of hooks.

From *N. bulbus, N. orchis, N. confuscus, N. acus* and *N. rubellus*, the present form can be distinguished in (1) the shape of the dorsal bar, (2) the differential development of hooks, (3) the nature of the cirrus and (4) the shape of the accessory piece.

It is evident, therefore, that the present form cannot be accommodated in the known species of the genus *Neodactylogyrus* Price, 1938. It is hence designated to new species which is named *N. cotius*, after the host.

REMARKS ON THE GENUS *Neodactylogyrus* PRICE, 1938

The genus *Neodactylogyrus* has an interesting history because as early as 1858, Diesing separated the species of *Dactylogyrus* with two bars as distinct from those with one bar, but did not name them separately. Price (1938) made a fission in the genus and recognised *Neodactylogyrus*—separate from *Dactylogyrus* Diesing, 1850. There is a great difference of opinion among various workers as to the validity of the genus *Neodactylogyrus*.

The genus *Neodactylogyrus* has been recognised by Price (1938), Kimpel (1939), Sproston (1946) and Yin and Sproston (1948), rejected by Mizelle and Donahue (1944), Monaco, Wood and Mizelle (1954), ignored by Gussev (1953) Mizelle and Klucka (1953) and Mizelle and Webb (1953).

A critical study of the subject reveals that the reasons given to synonymise the genus *Neodactylogyrus* with the genus *Dactylogyrus* are not very strong. It is remarked that the ventral bar is mostly vestigeal and undifferentiated from the dorsal bar but the study of the Indian species has revealed that both the bars could be clearly studied. The generic differences in the members of the family Dactylogyridae are mostly based on the differences in the chitinised armature of the haptor as well as that of the reproductive system. The present author firmly agrees with Price (1938) who made the fission in the genus *Dactylogyrus* on the character of the presence of one bar and two bars. Further, considering the large number of species, the genera *Dactylogyrus* and *Neodactylogyrus* have come to represent now, their recognition as separate genera would ease systematic study for future workers.

KEY TO THE INDIAN SPECIES OF NEODACTYLOGYRUS

1.	Cirrus thrown into loops	*N. cotius* n. sp.
	Cirrus not thrown into loops	2
2.	All hooks equally developed	*N. indicus* n. sp.
	All hooks unequally developed	*N. calbasi* n. sp.

SUMMARY

Three new species of the genus *Neodactylogyrus* Price, 1938 have been described from the gill filaments of some fresh-water fishes of Lucknow. The trematodes are *N. calbasi* n.sp. from *Labeo calbasu* (Ham); *N. indicus* n.sp. from *Puntius stigma* Cuv. & Val., and *N. cotius* n.sp. from *Rohtee cotio* (Bloch). This is the first record of this genus from Indian fishes. Remarks on the genus *Neodactylogyrus* have been given to support its generic generic validity. A key for the identification of the Indian species of *Neodactylogyrus* is appended.

ACKNOWLEDGMENTS

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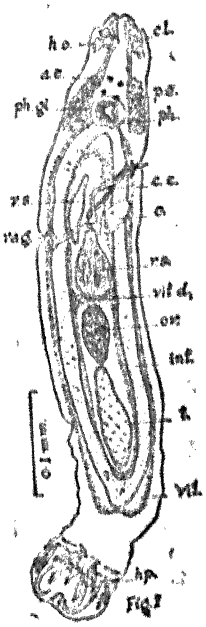

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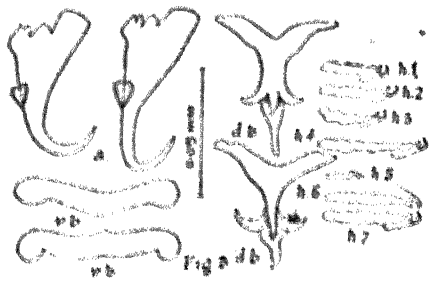

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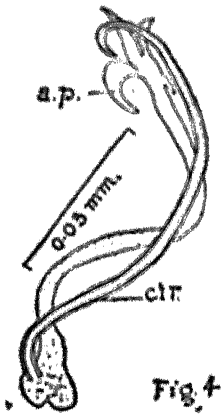

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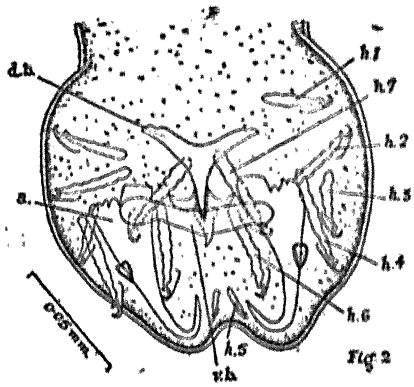

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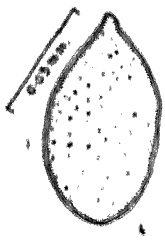

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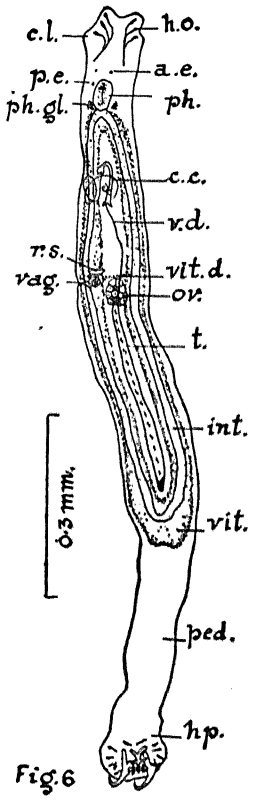

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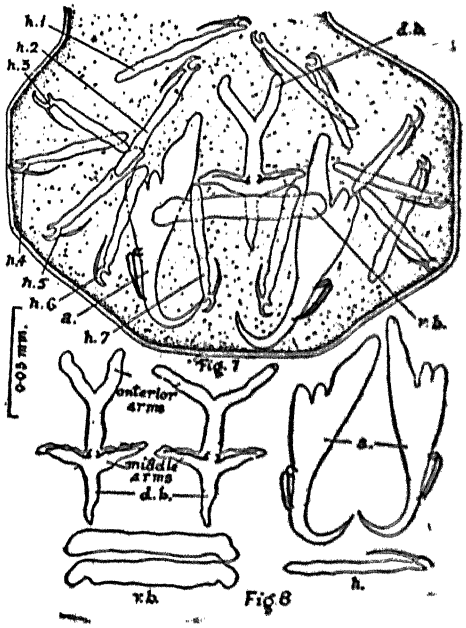
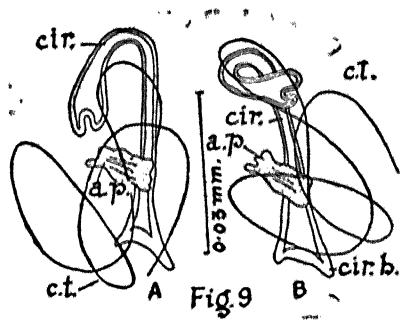


Fig. 7 Fig. 8



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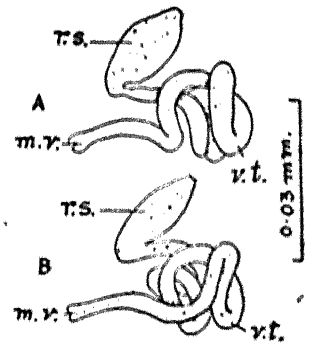

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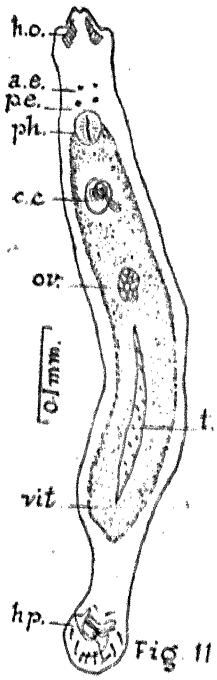

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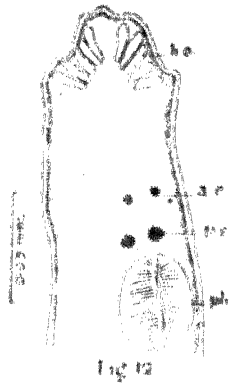
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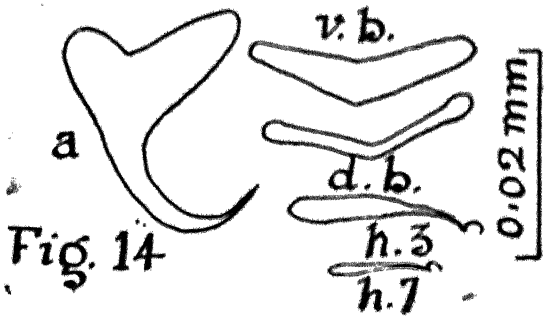

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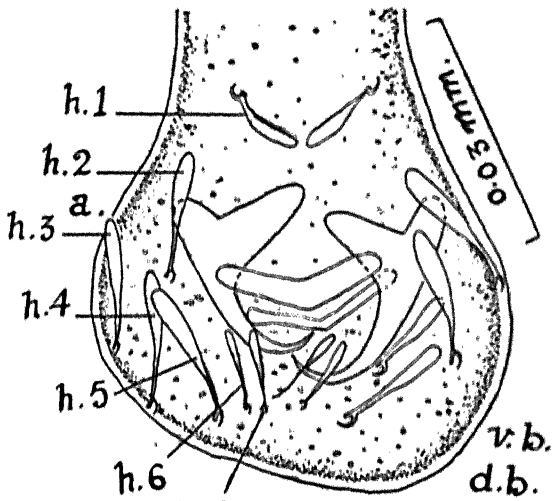

Fig. 13

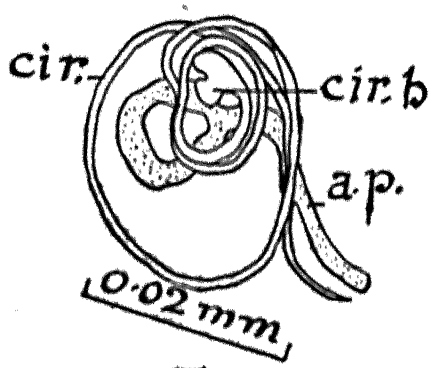

Fig. 15

EXPLANATION OF THE FIGURES

Fig. 1. *Neodactylogyrus calbasi* n.sp. Entire worm, dorsal view. a.e.—anterior eye-spots, c.c.—copulatory complex, c. l.—cephalic lobes, e.—egg, h.o.—head organ, hp.—haptor, int-intestine, ov.-ovary, p. e.-posterior eye-spots, ph.—pharyn, ph.gl.—pharyngeal glands, r.s.—receptaculum seminis, t.—testis, vag.—vagina, vit.-vitellaria, vit.d.-vitelline duct, v.s.-vesicula

Fig. 2. seminalis. *Neodactylogyrus calbasi* n.sp. Haptor, ventral view. a.—anchor, d.b.—dorsal bar, h.l-h.7.—hooks number 1 to 7, v.b.—ventral bar.

Fig. 3. *Neodactylogyrus calbasi* n. sp. Elements of haptor. a.—anchor, d.b.—dorsal bar, h.l.-h.7.—hooks 1 to 7, v.b.—ventral bar.

Fig. 4. *Neodactylogyrus calbasi* n. sp. Copulatory complex. a.p.—accessory piece, cir.—cirrus.

Fig. 5. *Neodactylogyrus calbasi* n.sp. Egg.

Fig. 6. *Neodactylogrus indicus* n. sp. Entire worm, dorsal view. a.e.—anterior eye-spots, c.c.—copulatory complex, c.l.—cephalic lobe, h.o.—head organ, hp.-haptor, int.—intestine, ov.—ovary, p.e.—posterior eye-spots, ped.—peduncle, ph.—pharynx, ph.gl.—pharyngeal glands, r.s.—receptaculum seminis, t.—testis, vag.—vagina, v.d.—vas deferens, vit.—vitellaria, vit. d.—vitelline duct.

Fig. 7-8 *Neodactylogyryus indicus* n. sp. Haptor and its armature. a.—anchor. d.b.-dorsal bar, h.—hook, h.l.-h.7.—hooks number 1 to 7, v.b.—ventral bar.

Fig. 9. *Neodactylogrus indicus* n. sp. Copulatory complex (A & B) a.p.—accessory piece, cir.-cirrus, cir.b.—base of cirrus, c.t.—cirrhal thread.

Fig. 10. *Neodactylogyrus indicus* n.sp. Vagina (A and B). m.v.—mouth of vagina, r.s.—receptaculum seminis, v.t.-vaginal tube.

Fig. 11. *Neodactylogyrus cotius* n. sp. Entire worm, dorsal view. a.e.—anterior eye-spots, c.c.-copulatory complex, h.o. head organs, hp.-haptor, ov.—ovary. p.e.—posterior eye-spots, ph.-pharynx, t.-testis, vit.-vitellaria.

Fig. 12. *Neodactylogyrus cotius.* Anterior end. a.e.-anterior eye-spots, h.o.-head organ, p.e.-posterior eye-spots, ph.-pharynx.

Fig. 13. *Neodactylogyrus cotius* n. sp. Haptor, ventral view. a.-anchor, d.b.—dorsal bar, h.l.-h.7.—hooks number 1 to 7, v.b.-ventral bar.

Fig. 14. *Neodactylogyrus cotius* n.sp. Elements of haptor a.—anchor, d.b.-dorsal bar, h. 3 & h. 7.—hooks number 3 and 7, v.b.—ventral bar.

Fig. 15. *Neodactylogyrus cotius* n.sp. Copulatory complex. a.p.—accessory piece, cir.-cirrus, cir.b.-cirrus base.

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